The ecological and evolutionary significance of reproductive traits in corals

UNIVERSITY
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Sebastian Schmidt-Roach
Institute for Marine and Antarctic Studies
University of Tasmania

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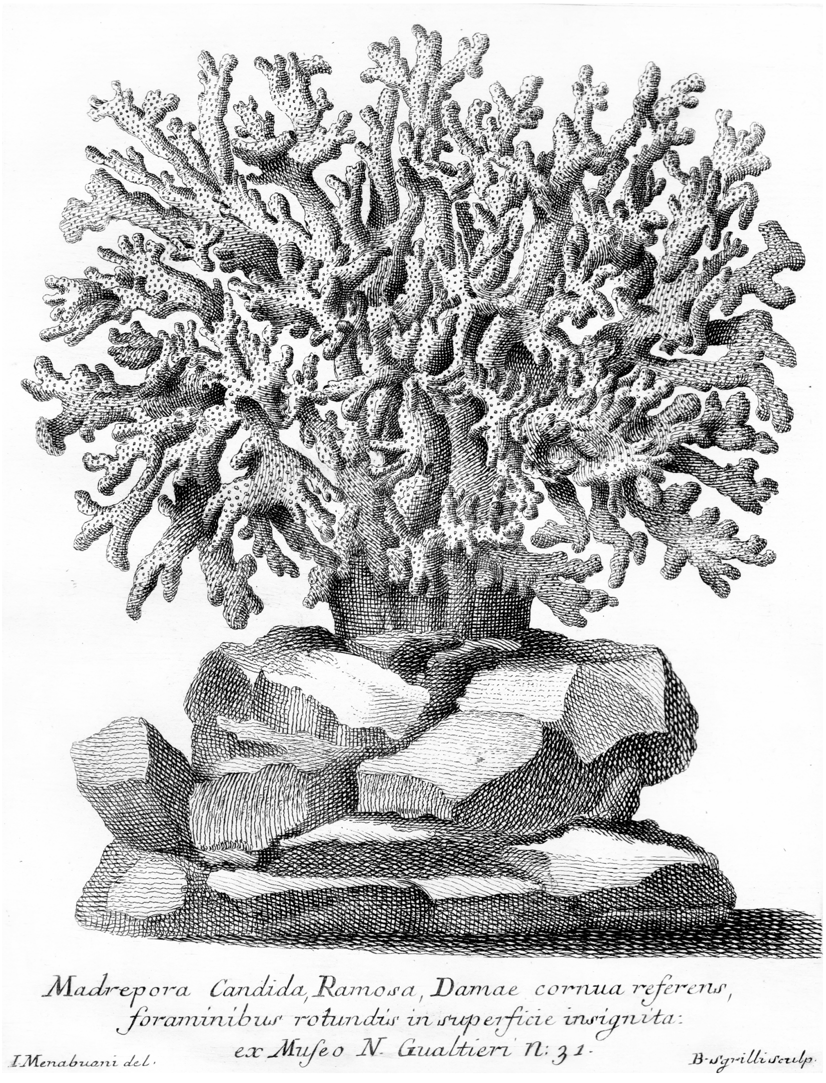

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THIS THESIS WAS SUPERVISED BY:

DR. KAREN J. MILLER, Institute for Marine and Antarctic Studies

DR. PETRA LUNDGREN, Great Barrier Reef Marine Park Authority

PROF. DR. GABRIELE GERLACH, University of Oldenburg

DR. NIKOLAOS ANDREAKIS, Australian Institute of Marine Science

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Signed: ____________________

Sebastian Schmidt-Roach
CANDIDATE

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OF MARINE SCIENCE

Statement of Co-Authorship

The following people and institutions contributed to the publication of work undertaken as part of this thesis:

Sebastian Schmidt-Roach, Institute for Marine & Antarctic Studies, University of Tasmania & Australian Institute of Marine Science = Candidate
Karen J. Miller, Institute for Marine & Antarctic Studies, University of Tasmania = Author 1
Petra Lundgren, Great Barrier Reef Marine Park Authority & Australian Institute of Marine Science = Author 2
Gabriele Gerlach, Carl von Ossietzky University of Oldenburg = Author 3
Nikos Andreakis, Australian Institute of Marine Science = Author 4
Annika M. E. Noreen, Australian Institute of Marine Science = Author 5
Erika Woolsey, Australian Research Council Centre of Excellence for Coral Reef Studies, James Cook University, Townsville, Queensland, Australia = Author 6
Andrew H. Baird, Australian Research Council Centre of Excellence for Coral Reef Studies, James Cook University, Townsville, Queensland, Australia = Author 7

Author details and their roles:

Paper 1, Assessing hidden species diversity in the coral *Pocillopora damicornis* from Eastern Australia. (2012) *Coral Reefs* pp. 112 DOI: 10.1007/s00338-012-0959-z
Located in chapter 2
Candidate was the primary author and conducted all experiments. Authors 1-4 contributed to the idea, its formalisation and development. Candidate conducted the data analysis, assisted by advice provided by authors 1-4. Author 5 contributed samples. All authors reviewed and commented on the final manuscript before submission.

Paper 2, Broadcast spawning by *Pocillopora* species on the Great Barrier Reef. (2012) *PloS ONE* 7:e50847
Located in chapter 3
Candidate was the primary author and authors 1 contributed to the idea, its formalisation and development. Author 1, author 3, author 6 and author 7 assisted during fieldwork. Candidate conducted the data analysis. All authors reviewed and commented on the final manuscript before submission.

Paper 3, *Pocillopora aliciae*: a new species of scleractinian coral (Scleractinia, Pocilloporidae) from subtropical Eastern Australia. (2013) *Zootaxa* 3626 (4):576582
Located in chapter 4
Candidate was the primary author and conducted all data collection. Author 1 and author 4 contributed to the idea, its formalisation and development. Candidate conducted the data analysis. All authors reviewed the final version before submission.

Paper 4, With eyes wide open: A revision of species within and closely related to the *Pocillopora damicornis* species complex (Scleractinia; Pocilloporidae). (Submitted) *Zoologial Journal of the Linnean Society of London*
Located in chapter 5
Candidate was the primary author and conducted all data collection. Author 1, author 2 and author 4 contributed to the idea, its formalisation and development. Candidate conducted the data analysis, assisted by advice provided by author 1 and author 4. All authors reviewed the final version before submission.

We the undersigned agree with the above stated "proportion of work undertaken" for each of the above published (or submitted) peer-reviewed manuscripts contributing to this thesis:

Signed: ____________________ ____________________

Dr. Karen J. Miller	Prof. Craig Johnson
Supervisor	Head of School
IMAS	IMAS
University of Tasmania	University of Tasmania

Date: ____________________

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Abstract

In this thesis, I explore species boundaries in corals and I investigate how distinct reproductive strategies may influence gene flow within and among species and ultimately how this affects processes of genetic isolation and speciation in corals.

Reconstructing genealogical relationships among colonies of pocilloporid corals sampled along Eastern Australia in chapter 2 confirmed the hypothesis that the extensively studied species *P. damicornis* constitutes a cryptic species complex rather than a single reproductively and morphologically plastic species. Haplotype networks computed from two mitochondrial DNA regions (CR, ORF) recovered at least five genetically distinct mitochondrial lineages within *P. damicornis* corresponding to distinct morphotypes previously considered ecomorphs. In addition, brooding and spawning observations on the Great Barrier Reef (GBR), suggested that the ancestral reproductive strategy in *Pocillopora* is broadcast spawning, while asexual brooding seems to be an apomorphic trait, characteristic to a single genetic clade that maintains a mixed mode of spawning and brooding. This confirmed the hypothesis for chapter 3 that *P. damicornis* (or hidden species within) brood larvae in Eastern Australia which are solely asexual. Further, the brooding clade contained three genetic lineages; two of them occur in sympatry in the Great Barrier Reef (GBR) and maintain brooding periods at opposite lunar phases. These GBR lineages showed clear nuclear divergences (HSP70, ITS2) confirming reproductive isolation likely due to differences in reproductive timing. In contrast, other lineages within *Pocillopora* lacked sharp nuclear divergence despite belonging to different mitochondrial clades and exhibiting different reproductive traits, indicating that occasional hybridisation occurs even between distant, otherwise well-defined lineages.

Further investigations, including additional *Pocillopora* species from Australia, confirmed mitochondrial molecular phylogenies are congruent with groups based on gross-morphology and symbiont association, therefore reflecting species-level differentiation. Fine scale morphological variation, particularly the shape and type of columella, added additional support for the differentiation of genetic lineages and provided an excellent signature of the evolutionary relationships amongst them.

The apparent misinterpretation of taxonomical units within *P. damicornis* may explain the perceived variation in the ecology, biology and life history of this species across its range. In order to provide appropriate taxonomic units for future references, colonies genotyped from Eastern Australia were compared to type specimens and original descriptions, which allowed for the revision of the taxonomic status of eight closely related species to and within the *P. damicornis* species complex, including the description of two new taxa. This confirmed the hypothesis that genetic lineages identified within *P. damicornis* correspond to taxonomic morphospecies (Chapter 4 & 5).

Much of what we know about reproductive relationships, including the ability of species to hybridise or self-fertilise, stems from *ex situ* fertilisation experiments and relies on the assumption that laboratory trials adequately reflect processes that are occurring in natural environments. However, this has never been tested empirically. Given that reproductive relationships within and among species play such an important role in the evolution of species, it is critical that we fully understand the links between laboratory trials and real world processes in order to make accurate predictions about species relationships and population dynamics. In chapter 6 I assessed the nature of fertilisation *ex-* versus *in-situ* in the hermaphroditic broadcast-spawning coral *Goniastrea favulus*, using seven newly developed microsatellite markers to compare estimates of selfing and outcrossing under natural and experimental conditions. In particular, I tested for the hypotheses that self-fertilisation represents an important reproductive strategy in *Goniastrea favulus* and that *G. favulus* favours non-self over self-fertilisation in situations of sperm choice. In the presence of self- and non-self sperm, the *ex situ* experiments showed high levels of selfing in *G. favulus* (30%), consistent with the known ability of this species to self-fertilise. However genotyping larvae fertilised under natural conditions revealed low levels of selfing on the reef ($<4\%$), suggesting that the occurrence of selfing in this species may have been significantly overestimated in laboratory trials and that laboratory experiments may fail to reflect real world conditions and lead to a distorted perception of reproductive relationships within and among coral species. In particular, the importance of inter-specific hybridisation between some coral species gained from similar laboratory fertilisation trials may have been similarly overestimated.

Hybridisation has been thought to be an important aspect of coral reproduction and has been considered, in part, to explain blurred morphological species boundaries in many genera and consequently reticulate evolution has been considered central to coral speciation. However, conclusions from ex situ trials that hybridisation occurs commonly, and hence its significance for coral evolution, may well be misleading. Nonetheless, my genetic data on *Pocillopora* suggest that occasional introgressive hybridisation at evolutionary time scales is likely to occur between some coral species. Within the genus *Pocillopora* semi-permeable species boundaries facilitated by occasional gene-flow among taxa may be an important evolutionary mechanism and may well contribute to the adaptive potential of this group as it allows for the exchange of beneficial genetic information between species, e.g. genes supporting adaptation to new environmental conditions as a result of climate change. Genetic transfer between lineages may reduce the individual risk of extinction and accelerate evolutionary rates leading to a higher relative potential for adaptation. Consequently, evolutionary plasticity facilitated by variation in reproductive traits may raise hope for the persistence of corals facing future climate change.

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ONE

INTRODUCTION

1.1
BACKGROUND

Coral reefs are among the most biologically diverse and economically important ecosystems on the planet and many communities rely on the services they provide for coastal protection, fisheries and tourism. Covering less than one tenth of one percent of the ocean floor (Spalding et al., 2001), they provide habitat to 32 animal phyla (in comparison, only 9 are found in terrestrial environments) (Wilkinson, 2002). Indeed, an estimated $5.5 billion of global net benefit is attributed to services provided by coral reefs (Cesar et al., 2003) and the Great Barrier Reef (GBR) in Australia alone is valued at $51.4 billion (Oxford-Economics, 2009). However, the number and intensity of threats to the biodiversity of coral reef ecosystems have dramatically increased over the last few decades (e.g. Hughes et al., 2003; Hoegh-Guldberg et al., 2007). For example, the coral cover of the Great Barrier Reef, one of the most extensively managed and studied reef systems, has decreased by 50.7% in the last 27 years (De'ath et al., 2012). In addition, it is predicted that coral-dominated ecosystems may further decline or be lost as a result of climate change (e.g. Hoegh-Guldberg et al., 2007). One the other hand, coral reefs survived significant climatic fluctuations in the past 240 million years responsible for periods of growth and decline. The apparent persistence of coral reefs suggests that reef constituents such as corals may be highly resilient, yet the adaptive potential of these organisms facing the current rapid climate change remains controversial (Nyström et al., 2000; Hoegh-Guldberg et al., 2002; Hughes et al., 2003; Baker et al., 2004).

Reef recovery following disturbances depends highly on recolonisation, thus understanding the life history of reef-building organisms is vital in order to predict levels of resilience (Pearson, 1981). A high diversity of life cycles has evolved, ranging from different strategies of larval production to different larval behaviours, yet the benefits and disadvantages of life histories in respect to connectivity and local adaptation remain poorly understood. From an ecological perspective, for most sessile marine invertebrates the pelagic larvae stage plays a vital role in their life cycle as it presents the only motile phase. Understanding the ecological significance that different life history strategies have on the structure and connectivity of populations may therefore provide information on the resilience (Ridgway, 2005). Further, from an evolutionary perspective, understanding how reproductive traits may influence the gene flow within and between metapopulation lineages may elucidate processes of speciation. Ultimately, illuminating these fundamental evolutionary mechanisms may reveal the source of the high adaptation potential corals have exhibited in the past 240 million years.

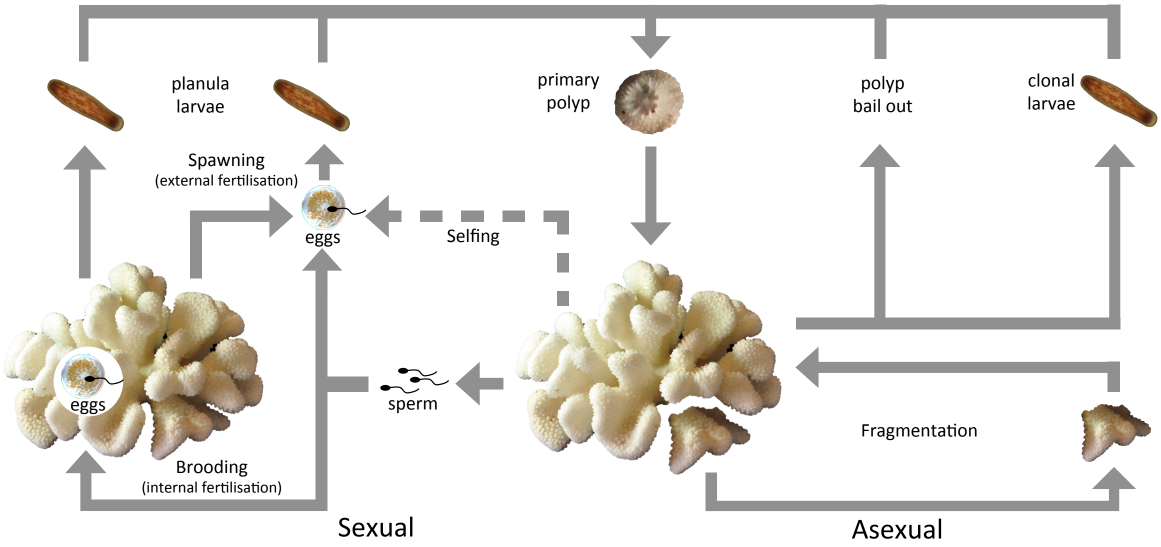


Figure 1.1: Schematic illustration of coral reproduction strategies.

1.1.1 Reproductive strategies and their significance

Understanding the adaptive significance of life history variation is a fundamental problem in evolutionary biology (Carlon, 1999). The reproductive mode and larval development are thought to have a significant effect on larval dispersal, gene flow and population structure (e.g. Ayre et al., 1997; Ayre & Hughes, 2000; Nishikawa & Sakai, 2005). Containing an extraordinary diversity of life-history variation (Fig. 1.1) at the species, population and individual level, corals represent ideal target organisms to study the importance of reproductive strategies in terms of ecology and evolution. In many species, both sexes are expressed within a single individual (simultaneous hermaphroditism). However, gonochorism (separate sexes) and sequential hermaphroditism also occurs, where, some corals exhibit the ability for multiple sex changes (Loya & Sakai, 2008). In addition to sexual reproduction, some corals reproduce asexually either through asexual reproduction of larvae, polyp bail out, pedal laceration or fragmentation (Highsmith, 1982; Sammarco, 1982; Stoddart, 1983; Harrison & Wallace, 1990).

Corals either brood larvae that are competent to settle upon release from the parent (Fig. 1.2), or spawn eggs and sperm for external fertilisation into the water column associated with a pelagic larvae development (Fig. 1.4). Brooding may occur through the internal fertilisation and development of eggs through conspecific sperm or clonal by parthenogenesis (Harrison, 2011). Furthermore, larval behaviour differs among species as some larvae show positively buoyant swimming, while others perform negatively buoyant swimming or benthic crawling (see Harrison & Wallace, 1990).

1.1.2 Sex, sperm dispersal and fertilisation

In contrast to terrestrial ecosystems, marine environments lack pollinators and sperm dispersal seems to be limited (see Levitan & Petersen, 1995). The decrease of sperm concentration due to dilution over distance appears to result in lower fertilisation rates, making fertilisation success dependent on population density (e.g. Levitan et al., 1992; Levitan & Petersen, 1995). Sperm dispersal in coral species is still undetermined, however, most studies conducted on other marine invertebrates indicate that sperm dispersal and thus successful fertilisation is limited to small scales. While successful fertilisation in the marine

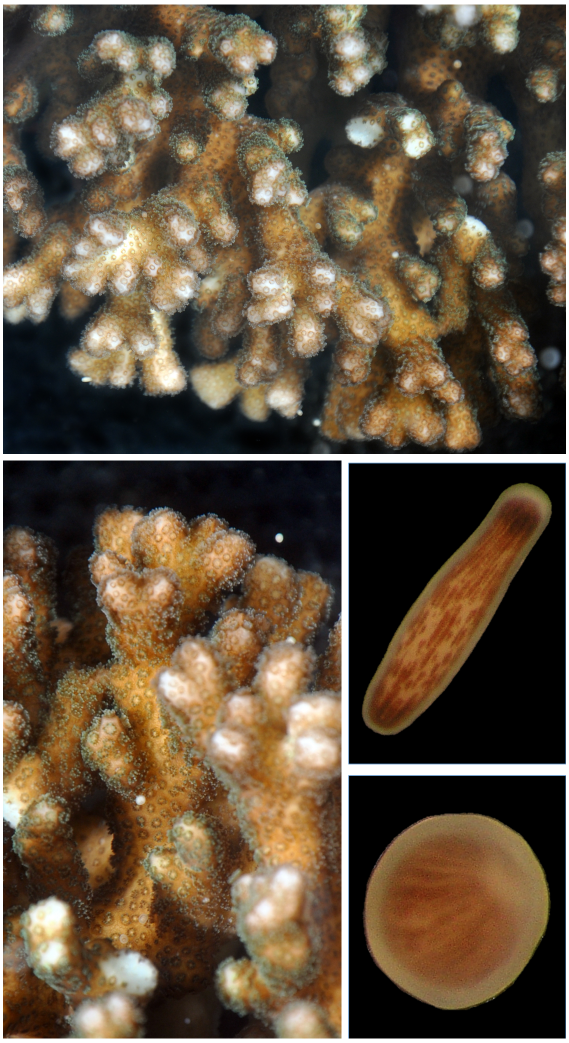


Figure 1.2: *Pocillopora damicornis* brooding, planula larvae (right)

hydroid *Hydractinia echniata* was observed on a scale of up to 10 meters with the majority occurring within three meters (Yund, 2005), the brooding demosponge *Crambe crambe* showed a mean dispersal distance of just 35 cm (Calderon et al., 2007). In contrast, sperm dispersal in the asteroid *Acanthaster planci* is reported to be over 100 m downstream (Babcock et al., 1994). Different mechanisms have evolved to increase fertilisation and reproductive success in marine benthic populations. While brooding species may maintain unfertilised eggs for days or even weeks, species that spawn gametes for external fertilisation depend highly on synchronous release (Harrison & Wallace, 1990).

In addition to synchronous spawning, many broadcast-spawners release positively buoyant egg-sperm-bundles, which break apart and accumulate at the sea surface (Oliver & Babcock, 1992). This behaviour is believed to contribute to higher sperm concentrations and better chances of mixing with conspecific sperm/eggs (Oliver & Babcock, 1992). From an evolutionary perspective marine sessile organisms such as corals face a tradeoff. While philopatric recruitment in approximate distance to conspecific may increase fertilisation rates, it will ultimately increase the chance of settling next to relatives and thus may lead to significant inbreeding (i.e. the mating of closely related individuals) (Grosberg, 1987). Inbreeding is generally associated with the loss of genetic diversity and may lead to inbreeding depression, the reduced survival and fertility of offspring of related individuals (Charlesworth & Charlesworth, 1987). Small, isolated populations are especially exposed to inbreeding, which can cause substantial fitness reductions compared to outbred populations (Keller & Waller, 2004). Indeed, many genetic studies in corals detected high heterozygosity deficits commonly explained by inbreeding due to restricted larvae dispersal (Whitaker, 2004; Miller & Ayre, 2008a; Combosch & Vollmer, 2011). The loss of fitness due to inbreedings has been predicted to increase extinction risk giving it substantial conservation significance (Wright et al., 2008). Determining the rates of self-fertilisation, inbreeding and outbreeding of coral populations is therefore of high interest to understand the evolutionary processes influencing the persistence of a species.

1.1.3 Reproduction in the absence of conspecifics

Similar to plants, most corals have the ability to reproduce asexually via fragmentation (Harrison & Wallace, 1990). In contrast to fragmentation, reproduction strategies such as self-fertilisation

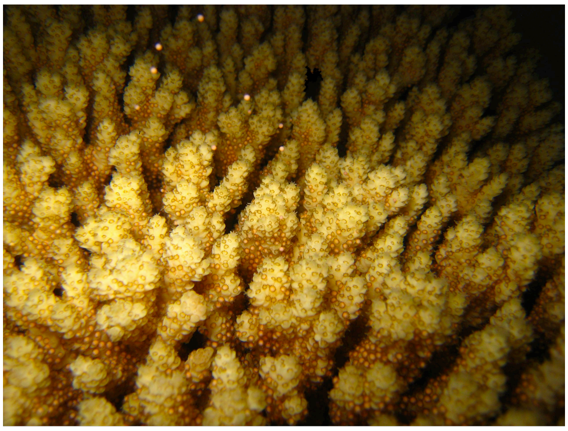
Figure 1.3: *Goniastrea favulus* spawning sperm, after eggs–bundles have been released.

(Stoddart et al., 1988), polyp bail out (Sammarco, 1982) or the brooding of clonal larvae (Stoddart, 1983; Ayre & Miller, 2004; Sherman et al., 2006) allow the genotype to return into a planktonic life stage (Fig. 1.4). In situations were conspecifics are out of reach, returning to a motile larval stage may assist the genotype to disperse and ultimately escape isolation. Self-fertilisation guarantees sexual reproduction and may be particularly beneficial in situations of low abundance such as after catastrophic events or after arrival in a new system, where close conspecifics are absent (Heyward & Babcock, 1986). Yet, Darwin (Darwin, 1876) already noted that in plant experiments self-fertilisation is strongly disadvantageous for the production of offspring in most species. Barriers to self-fertilisation seem to have evolved in most species to favour out-crossing, although these barriers may break down over time allowing selfing if out-crossing fails (Heyward & Babcock, 1986). Little is known about the advantages and disadvantages of selfing in corals. Fertilisation trails suggest that selfing rates may be high in the self-compatible, broadcast-spawning coral *Goniastrea favulus* (Stoddart et al., 1988). In addition, natural populations of the brooding corals *Favia fragum* and *Porites astreoides* showed high levels of selfing. Studies on other marine invertebrates like the colonial, simultaneous hermaphrodite bryozoa *Bugula stolonifera* selfing show that selfing leads to significantly decreased survival and reduced reproductive output (Johnson, 2010). However, in *B. stolonifera* high settlement aggregation of non-kin larvae seems to have evolved to increase outbreeding (Johnson, 2010). Thus, certain aspects of the life history, such as specific larvae behaviour, may counteract and regulate otherwise disadvantaged strategies.

Similar to self-fertilisation, asexual brooding allows for multiplication in the absence of conspecifics. Stoddart (1984b) found a few clones of the coral *Pocillopora damicornis* to dominate some populations in Western Australia. This dominance of certain well-adapted clones should influence the population structure, leading to an increase of inbreeding (Knowlton & Jackson, 1993). However, little is known of the contrasting roles that sexual and asexual reproduction play within these complex life-cycles (Ayre & Miller, 2004). Understanding the benefits or disadvantages of each life history in respect to connectivity and local adaptation is of great interest as it may answer important questions on abundance, potential for recolonisation of disturbed areas and resilience of these species.

1.1.4 Dispersal

Larval dispersal, and therefore migration, is dependent on a variety of parameters, such as the pre-competency period, survival rate of competent larvae, larval behavior (active swimming, benthic crawling), oceanographic factors (current patterns) transporting the larvae and the availability of suitable substrate for settlement and recruitment (Harrison & Wallace, 1990). The apparent lack of conventional barriers in marine environments as well as planktonic larval stages of benthic organisms led to the assumption that dispersal should be relatively unrestricted and



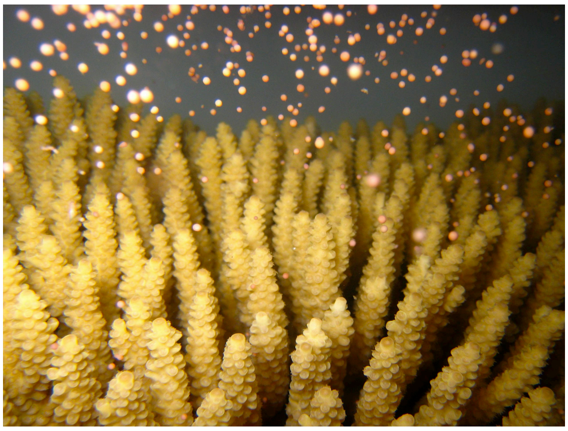


Figure 1.4: Top: *Acropora tenuis* starting to spawn - polyps are setting sperm-egg-bundles for synchronous release, first bundles are dribbling to the surface. Bottom: *Acropora millepora* during spawning.

that marine populations should be characterised by high levels of gene flow over vast geographical areas (Harrison & Wallace, 1990). As direct tracking and observation of larvae *in situ* is impossible for most invertebrate species due to their small size (Harrison & Wallace, 1990), dispersal potential is often inferred from ocean currents and *ex situ* competency experiments (Harii et al., 2002). The application of molecular tools has made it possible to indirectly access levels of connectivity and dispersal and increasing numbers of empirical studies have shown significant genetic subdivision in corals (e.g. Underwood et al., 2007; Ridgway et al., 2008) and restricted larval dispersal across multiple spatial scales, thus contradicting the assumed panmixia over vast geographical distances. However, where stepping stones such as intermediate reefs exist, studies indicate limited genetic differentiation (hence high connectivity) (Ayre & Hughes, 2000; Ridgway et al., 2001; Baums et al., 2005; Magalon et al., 2005) and genetic panmixia at relatively small spatial scales such as tens of kilometres or less (Oppen & Gates, 2006). In addition, high to moderate genetic structures at local scales coupled with apparent gene flow over larger scales are typical for coral populations (e.g. Ayre & Hughes, 2000; Magalon et al., 2005). Indeed, only few migrants are needed to genetically connect populations (Hellberg, 1995), which may explain why only few studies indicate isolation by distance (Oppen & Gates, 2006).

1.1.5 Long versus short distance larva dispersal

Long distance dispersal is dependent on the larva survivorship and settlement competency. Brooders are generally expected to survive long-distance dispersal as most larvae possess symbiotic algae from which they may gain energy (Richmond, 1987), e.g. brooded larvae of *Pocillopora damicornis* have a competency period of about 100 days (Harii et al., 2002). Nevertheless, some broadcast-spawners like *Pocillopora verrucosa* also contain symbiotic algae (Sier & Olive, 1994). In addition, broadcasted coral larvae lacking zooxanthellae are able to survive similar periods (Graham et al., 2007). Consequently long distance dispersal might not be that different between both life history strategies. Furthermore, high larval mortality in the early stages seems to decrease the survival rate of larvae transported to neighbouring reefs and thus decreases the connectivity of the region (Graham et al., 2007). This variation in the larval survivorship based on age may explain why high genetic structure is found at metapopulation scales, coupled with the maintenance of extensive geographic ranges observed in many coral species (Graham et al., 2007). Nevertheless, extremes of long distance dispersal may be achieved by rafting on floating objects, which is described for different marine invertebrate including corals (Jokiel, 1984; Highsmith, 1985).

As brooded larvae are typically competent immediately after release, they are expected to settle locally to a greater extent than broadcast-spawners (Harrison & Wallace, 1990). Therefore, brooding may result in a patchy distribution with more genetic substructure than in broadcasting species (Benzie et al., 1995; Brazeau et al., 2005). High genetic local variability was discovered for the brooding pocilloporid coral *Seriatopora hystrix*; indicating that populations are locally subdivided and the majority of larvae settled within 100 m of their natal colony (Maier et al., 2005; Underwood et al., 2007). In addition, a study on *Acropora tenius* and *Stylophora pistillata* using allozymes supported the prediction that neutral genetic differentiation is greater among populations of brooders compared to broadcast spawners (Nishikawa et al., 2003). However, settlement timing of brooders and broad- cast spawning species may not differ greatly (Miller & Mundy, 2003). Indeed, several studies have found limited dispersal and strong local population structures even for spawning species (Miller & Ayre, 2008a; Combosch & Vollmer, 2011). Thus, the impact of different life history strategies and larval development on the distribution remains unclear. Nevertheless, many findings indicate that dispersal at ecological time scales seems to be restricted, creating subdivided metapopulations.

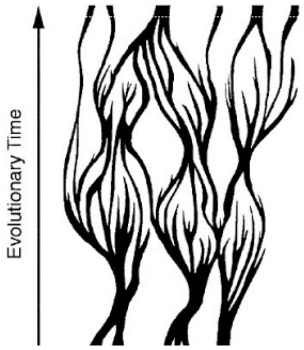
1.1.6 Outbreeding and hybridisation

In contrast to inbreeding, outcrossing, including the extreme of interspecific hybridisation (the mating of individuals of two different gene pools or species) may help to enrich the gene pool (Willis et al., 2006). At the population level, outcrossing of local genotypes with successful long distance dispersing genotypes of different populations/gene pools may counteract inbreeding effects or genetic depression and introduce new genetic diversity. Indeed, genetic studies of remote and isolated high latitude systems indicate that low recruitment of migrants on rare occasions (>1 migrant per generation) has the potential to counteract the loss of genetic variation through genetic drift and connect even distant populations (Nunes et al., 2008; Noreen, 2010; Nakajima et al., 2012).

Experiments suggest that hybridisation among morphologically distinct coral species may be common (Miller & Babcock, 1997; Willis et al., 1997; Márquez et al., 2002; Willis et al., 2006) and at least possible at genus level, e.g. *Platygyra* and *Leptoria* (Miller, 1994a). Indeed, genetics indicate that some rare *Acropora* species found in remote systems likely represent inter-species hybrids (Richards et al., 2008). In the Caribbean extensive introgressive hybridisation between *Acropora cervicornis* and *A. palmata* is thought to create a morphological distinct hybrid species, *A. prolifera*, which backcrosses with the parental species at low frequency (Oppen et al., 2000). However, most species seem to have some pre-zygotic mechanisms that prevent hybridisation. Nevertheless, synchrony in timing of reproduction, such as the synchronous gamete release of multiple species during annual spawning events, is essential to allow hybridisation (Babcock, 1995; Willis et al., 1997). Paradoxically, although synchronous spawning events should promote interspecific hybridisation, species diversity within scleractinian corals is high (Vollmer & Palumbi, 2002), thus natural hybridisation seems to be limited in most species (Veron, 1995). Indeed, a recent study on acroporids in Taiwan showed that most species dont spawn exactly at the same time and that if rare cross-fertilisation occurs, post-fertilisation mortality of hybrids is high (Wei et al., 2012). Similar findings were made for species within the genus *Platygyra* (Miller & Babcock, 1997). Furthermore, closely related species of siphonous green seaweed (Bryopsidales) have been found to release gametes at the same morning, but at differ-

Recticulate evolution

Reticulation describes the merging of two divergent species into one species through hybridisation. The concept of reticulate evolution, first applied to corals by Veron (1995), acknowledges that the independence of a lineage/species may be temporal. Driven by environmental or spatial changes, separately evolving lineages may reticulate and merge by hybridisation. Thus the evolutionary history of a species may be derived from ancestral lineages which were at a certain point in time separated from one another.



Right: Illustration of reticulate evolution by Veron (1995).

ent times, preventing hybridisation (Clifton, 1997; Clifton & Clifton, 1999). In contrast to spawning species, the time window for fertilisation may be larger in brooding species as unfertilised eggs are potentially stored over several days (Veron, 1995). However, apparent hybrids between *Stylophora pistillata* and *Pocillopora damicornis* identified at Lord Howe Island (Miller & Ayre, 2008b) and hybrids between Caribbean *Madracis* species (Frade et al., 2010) are examples identified among brooding species.

Phylogenetics (the study of evolutionary relationships among organisms based on divergences in molecular sequences) have been of great assistance to detect cases of natural hybridisation, however, the lack of monophyletic divergence between species can be equally explained by high phenotypic plasticity of a single species, incomplete lineage sorting or introgressive hybridisation (see Willis et al., 2006). Introgressive hybridisation describes the repeated backcrossing of an inter-specific hybrid with one of its parent species and thus the directional gene flow from one species into the gene pool of another. Rather than resulting in a distinct hybrid species with intermediate characteristics to the parental species, prolonged introgressive hybridisation may ultimately result in reticulate evolution (Veron, 1995; Willis et al., 2006) (see box). However, occasional introgressive hybridisation may be beneficial and increase the adaptive potential of a species though the transfer of genetic information from a locally well adapted species (Seehausen, 2004).

1.1.7 Life history and speciation

Life history may play an important role in the processes of speciation. Several stages of life history may play key roles in driving isolation of populations. These include (1) larvae dispersal competency, (2) larval behaviour, and (3) time of reproduction. Considering that dispersal seems to be more limited than previously considered, the speciation potential of benthic marine taxa may have been significantly underestimated (Knowlton & Jackson, 1993). Indeed, cryptic speciation identified in the ascidian *Pycnoclavella communis* seems to be attributed to short-distance dispersal of larvae resulting in restricted gene flow among populations and high levels of local genetic differentiation (Perez-Portela & Turon, 2008). Limited dispersal leading to isolation of subpopulations may be a significant driver of evolution for some species and explain why especially remote, isolated subtropical systems seem to accommodate evolutionary novelty (Budd & Pandolfi, 2010). In addition to mechanisms of isolation by distance, allopatric evolution in the sea may also be driven by adaptation to temperature, salinity or turbulence regimes all providing environmental barriers.

Aspects of larvae behavior include cues of settlement and metamorphosis. Differences in settlement preferences may lead to habitat specialisation and limit interbreeding of populations as observed for the *Mytilus edulis* species complex (Bierne et al., 2003). Specificity to certain cues triggering metamorphosis and settlement is well known for several coral species (e.g. Harrington et al., 2004; Golbuu & Richmond, 2007) and may be a significant driver for niche adaptation.

Differences in time of reproduction, i.e. a lack of synchrony of reproduction may lead to divergence of populations over time. Temporal divergences in the scale of hours from mass spawning events have been hypothesised to cause speciation of some species ((e.g. *Acropora*, Fukami et al., 2003, Wei et al., 2012; *Montastrea*, Levitan et al., 2004).

In Taiwan intra-specific reproductive isolation was identified for populations of *Mycedium elephantotus* that maintains populations that reproduce at opposite seasons (Dai et al., 2000), a finding that could also be observed in Western Australia for populations of *Acropora samoensis* and *A. cytherea* (Rosser & Gilmour, 2008). Further, reproductive isolation due to asynchronous planulation at opposite lunar cycles has also been proposed for populations of the brooding species *Pocillopora damicornis* in Hawaii (Richmond & Jokiel, 1984).

1.1.8 Hybrids and systematics

These previously named mechanisms associated to different life history stages may drive the speciation processes, however, not all marine invertebrate species may be isolated based on strict gamete incompatibility or post mating sexual selection as previously stressed. Thus separately evolving metapopulation lineages may be weakly connected by occasional introgressive hybridisation or what is considered secondary contact between species. Hybrids arise where isolating mechanisms fail and indeed, the lack of strict reproductive isolation and clear molecular monophyly due to occasional hybridisation seems to encrypt many coral species boundaries and thus often prevents the use of conventional species concepts such as the phylogenetic species concept (Cracraft, 1987) or the biological species concept (Mayr, 1963). In contrast to ordinary speciation, evolutionary novelty in hybrids arises from a novel genetic recombination of existing entities due to a disruption of the divergence process of two species (Barrington et al., 1989). Consequently, conventional species concepts often fail to classify hybrid species such as *A. prolifera* (see Oppen et al., 2000), although these groups may be characterised by individual features or habitat preferences giving these groups an ecological significance.

1.2

AIMS OF THESIS

1.2.1 Overall objective

This thesis investigates the evolutionary and ecological roles of different reproductive strategies and their impact on population structure. Two coral species are investigated with contrasting life histories, the generally brooding coral species *Pocillopora damicornis* and the broadcast-spawning species *Goniastrea favulus*. Due to numerous studies (including population genetics) conducted on these species, both present optimal target species for the further examination of the link between life history and population structure (*P. damicornis*: e.g. Stoddart, 1984b; Benzie et al., 1995; Ayre et al., 1997; Ayre & Hughes, 2000; Miller & Ayre, 2004; Sherman et al., 2006; Souter, 2010; *G. favulus*: e.g. Stoddart et al., 1988; Miller & Mundy, 2005; Miller & Ayre, 2008a). Although contrasting in their mode of reproduction, both species share strategies, which guarantee reproduction in the absence of conspecifics. While *P. damicornis* is known to produce clonal broods, *G. favulus* is known to self-fertilise. Furthermore, both have larval development that allow for a philopatric recruitment. Understanding the significance of these strategies may further elucidate their evolutionary roles in these complex life histories and add valuable information about the resilience of these species to environmental changes such as future climate change scenarios.

1.2.2 *Pocillopora damicornis*

The first target species, *Pocillopora damicornis* (Linnaeus 1758), is a widely distributed and extensively studied hermaphroditic reef coral with a complex and yet unclear life history. *P. damicornis* is described to exhibit an extraordinary variability in its life history with different reproductive modes and varying times of reproduction within and between seasons depending on its geographic location. This species is generally described as a brooder, however, it is known to reproduce via spawning in the Tropical Eastern Pacific (TEP) (Glynn et al., 1991; Rodríguez-Troncoso et al., 2011) as well as suggested to harbour a mixed mode of spawning and brooding in Australia (Muir, 1984; Ward, 1992). Although brooding has been confirmed in several studies (Marshall & Stephenson, 1933; Harriott, 1983b; Stoddart, 1983; Muir, 1984; Ward, 1992; Ayre & Miller, 2004), spawning has only been inferred from histology. In the Great Barrier Reef (GBR) the taxon has been described to be a brooder of exclusively asexual larvae (Ayre & Miller, 2004; Sherman et al., 2006), however, mixed broods of clonal and partial sexual larvae have been reported in Taiwan (Yeoh & Dai, 2010). In contrast to its clonal mode of reproduction, *P. damicornis* populations on the GBR typically exhibit high levels of genotypic diversity and low levels of genetic differentiation, which is consistent with predominantly sexual reproduction and recruitment (Benzie et al., 1995; Ayre et al., 1997; Ayre & Miller, 2004; Miller & Ayre, 2004; Sherman et al., 2006), thus the reproductive efforts and genotypic diversity of local populations appear to be mismatched. Sherman et al. (2006) showed that asexually brooded larvae of *P. damicornis* did not contribute significantly to maintaining local populations in the majority of reef habitats at OTI. However, clones dominated three habitats within OTI which were affected by major disturbances (1998 bleaching event) (Sherman et al., 2006). Interestingly, one of the investigated micro atolls showed no indications of asexual reproduction in an earlier study (Benzie et al., 1995). This suggests that asexual recruitment may be favoured after a disturbance event, presumably when competition is reduced (Sherman et al., 2006). However, from an evolutionary perspective it is puzzling how *P. damicornis* can maintain genotypically diverse populations that appear to be solely sexually derived and still invest vast amounts of energy into a form of reproduction that is apparently not utilised (Ayre & Miller, 2004). Further, the high plasticity of reproductive strategies reported at different geographical locations prompts questions on what ecological factors are driving this variation.

1.2.3 Reproductive plasticity or cryptic speciation

Interestingly, genetic evidence indicates that *P. damicornis* from the Indian Ocean consists of two cryptic species with different population structures (Souter, 2010). This finding raised questions if some of the contradicting findings made for its population structure and reproductive mode may have resulted from hidden species diversity. Thus chapter 2 tested for the following hypothesis:

1.2.3.1 Hypothesis 1

> *P. damicornis* consists of a species complex rather than a single reproductively and morphologically plastic species.

1.2.4 The significance of asexual brooding

Although hidden species diversity is likely accountable for some of *P. damicornis* reported reproductive plasticity, the apparent mismatch between its clonal mode of reproduction and its seemingly sexually derived population structures still raised questions on the origin of sexual and the fate of asexual larvae. Thus, using an integrative approach combing phylogenetics with data on reproduction, gross morphology and symbiont association, chapter 3 tested for the following hypothesis:

1.2.4.1 Hypothesis 2

> *P. damicornis* (or hidden species within) brood larvae in Eastern Australia which are solely asexual.

1.2.5 Revision of species within the *P. damicornis* species complex

Due to findings of hidden species that harbour different reproductive strategies within chapter 2 & 3, the following two chapters (4 & 5) aimed to formally revise the taxonomic units identified and tested for the following hypothesis:

1.2.5.1 Hypothesis 3

> The genetic lineages identified within *P. damicornis* correspond to taxonomical morphospecies.

1.2.6 Sperm competition

Much of what we know about reproductive relationships in coral species stems from *ex situ* fertilisation experiments, and relies on the assumption that laboratory trials adequately reflect processes that are occurring on the reef. Given that reproductive relationships within and among species play such an important role in the evolution of species, it is critical that we fully understand the links between laboratory trials and real world processes in order to make accurate predictions about species relation- ships. Here I assess fertilisation rates *ex* versus *in situ*. Findings of this study will be of assistance to examine how comparable laboratory fertilisation trials are to real world situations in corals. This will help us to evaluate predictions made on fertilisation rates in corals, including those made on selfing or inter-specific hybridisation.

1.2.7 *Goniastrea favulus*

Due to the lack of knowledge about the spawning behaviour in *Pocillopora* species, I chose *Goniastrea favulus* (Dana, 1846), as a target species to study fertilisation rates. In contrast to *P. damicornis*, *G. favulus*, reproduces solely by spawning. However, being hermaphroditic and self-compatible (Heyward & Babcock, 1986; Stoddart et al., 1988) its shares the ability of reproduction in the absence of conspecific with *P. damicornis*. Rates of self-fertilisation have been hypothesised to be high (Stoddart et al., 1988), although a recent study found limited evidence of self-fertilisation based on its population structure (Miller & Ayre, 2008a). *G. favulus* releases gametes freely and has sticky, negatively buoyant eggs that remain attached to the parent colony through fertilisation (Quinn & Kojis, 1981; Heyward & Babcock, 1986; Miller & Mundy, 2005). Sperm is released subsequent to the release of eggs (Quinn & Kojis, 1981) (Fig. 1.3). Following spawning, the egg clumps gradually drift from the surface of the colony and sink, eventually adhering to the substrate in relatively close proximity to the parent colony (Quinn & Kojis, 1981).

High population densities and low water levels at the time of spawning may lead to high sperm concentration in the water and subsequently high fertilisation rates (Miller & Mundy, 2005). From an evolutionary perspective, if, the organism is a hermaphrodite and self-compatible, outcrossing should be favoured in the presence of conspecifics and mechanisms should be in place to avoid selfing (Heyward & Babcock, 1986). To test the significance of selfing in the presence of conspecific, chapter 6 tested for the following hypotheses:

1.2.7.1 Hypothesis 4

Self-fertilisation represents an important reproductive strategy in *Goniastrea favulus*.

1.2.7.2 Hypothesis 5

G. favulus favours non-self over self-fertilisation in situations of sperm choice.

CHAPTER

TWO

ASSESSING HIDDEN SPECIES DIVERSITY IN THE CORAL *POCILLOPORA DAMICORNIS* FROM EASTERN AUSTRALIA

2.1

INTRODUCTION

Inconsistent species identification leads to errors in biological diversity estimates and may result in confusing or misleading interpretations of ecological and evolutionary data (Bickford et al., 2007). With the advent of molecular tools, we are now in a position to better understand patterns of species diversity and distribution. Furthermore, initiatives such as the Barcode of Life (www.barcodeoflife.org) allow consistent identification of taxa through combining rigorous morphological taxonomy with DNA sequence data.

It is becoming apparent that many ecological studies have failed to account for the occurrence of cryptic speciation, even within iconic and well-studied terrestrial taxa such as the giraffe and the African elephant (Roca et al., 2001; Brown et al., 2007). In marine species such as scleractinian corals, high levels of morphological variation have confounded our understanding of species boundaries. In addition, molecular phylogenies of corals often fail to recover reciprocally monophyletic clusters supported by fixed morphological characters due to extensive hybridisation, ongoing speciation or unresolved ancestral polymorphisms (Oppen et al., 2001; Flot & Tillier, 2006; Willis et al., 2006; Souter, 2010). To accommodate more than one conspicuous morphotype within presumably distinct scleractinian taxa, Veron & Pichon (1976) introduced the concept of "ecomorphs" and suggested that much of the morphological variation previously attributed to species can be induced by environmental gradients. This phenomenon, previously described widely in terrestrial plants (Schlichting, 2008, and references therein), has been confirmed in corals by transplant experiments (e.g., Willis, 1985; Bruno & Edmunds, 1997; Todd, 2008).

The common reef-building coral *Pocillopora damicornis* (Linnaeus, 1758) is often considered the "poster child" of morphological plasticity among its tropical relatives. Its morphology ranges from a filiform, pointed, branching colony to a compact and stunted posture, depending on whether it is growing in wave-exposed or protected environments (Fig. 2.1), modified from Veron & Pichon 1976). Based on variations in gross morphology, Veron & Pichon (1976) synonymised five *Pocillopora* taxa as distinct ecomorphs of the presumably genetically homogeneous, yet highly plastic species *P. damicornis*. One additional morphotype, δ is a subtropical form that was only found in the Solitary Islands and is not depicted hereThe aforementioned classification, however, has proven largely inadequate to solve significant

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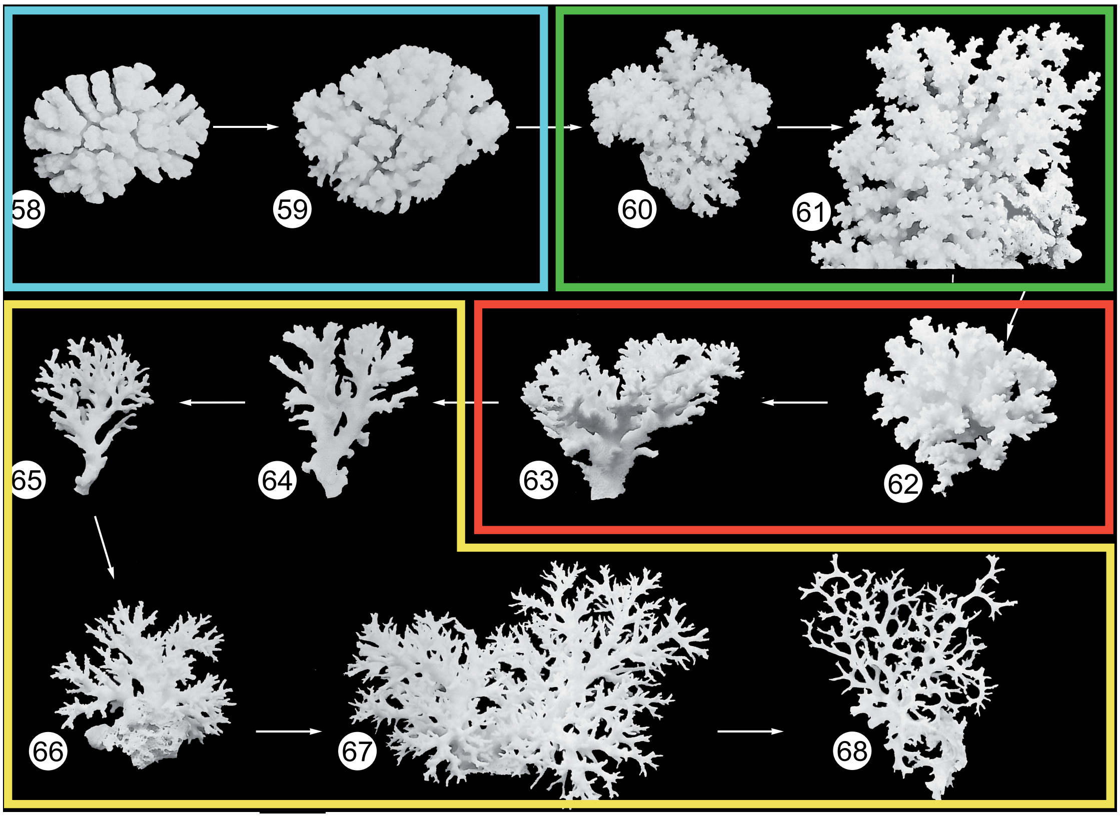


Figure 2.1: Ecomorphs of *Pocillopora damicornis* from the GBR, photographed and described by Veron & Pichon (1976). Coloured boxes indicated morpho-groups used in this study. α = green, β = yellow, γ = red, ϵ = blue

aspects of the species life history. For instance, *P. damicornis* has been described as either an asexual or sexual brooder, and/or a broadcast spawner throughout the ecomorphs reported distribution range, with variable timing of reproduction depending upon lunar phase and season (e.g., Harriott, 1983b; Richmond & Jokiel, 1984; Stoddart & Black, 1985; Ward, 1992; Tanner, 1996; Yeoh & Dai, 2010). Most importantly, although colonies from Australias Great Barrier Reef (GBR) are known to reproduce only via asexual brooded larvae, they display a population genetic structure typical of random-mating sexually reproducing species (Benzie et al., 1995; Ayre et al., 1997; Ayre & Miller, 2004; Miller & Ayre, 2004; Sherman et al., 2006). The latter incongruence challenges the distinct influence of sexual or asexual reproduction in the maintenance of marine populations genetic profile.

Recent molecular studies indicated that multiple genetically distinct lineages exist within *Pocillopora damicornis* (Combosch et al., 2008; Flot et al., 2008; Pinzón & LaJeunesse, 2010; Souter, 2010). It is therefore plausible that multiple genealogically independent lineages may have been included in earlier ecological studies, thus producing inconsistent or contradictory results (e.g., Ayre et al., 1997). Here, I test for evidence of hidden *Pocillopora* lineages or biologically distinct species within *P. damicornis* in Eastern Australia that might explain the intriguing extent of biological and ecological variation in this important reef-building coral. Specifically, I determine whether morphological groups previously suggested to be the result of local environmental conditions corroborate multiple valid taxonomic units within *P. damicornis*.

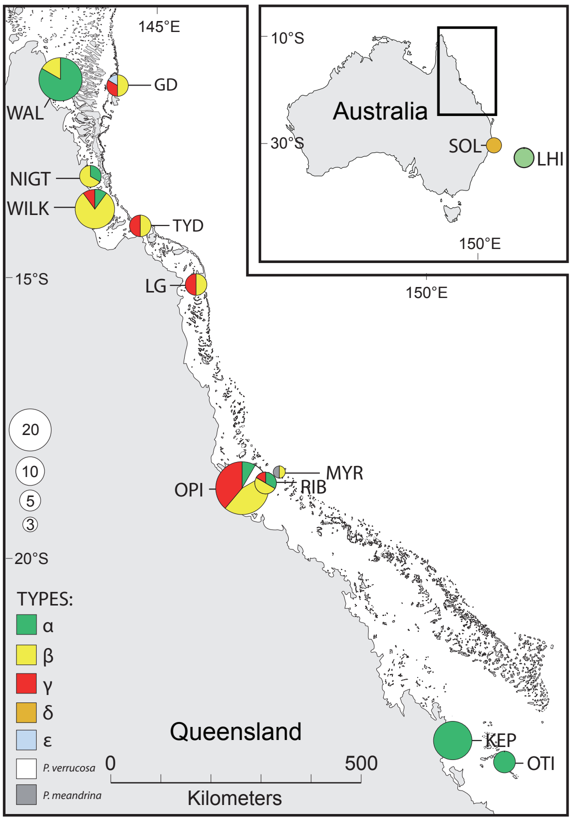


Figure 2.2: Specimen collection sites. Great Detached Reef (GD), Wallace Islet Reef (WAL), Night Reef (NIGT), Wilkie Reef (WILK), Tydeman Reef (TYD), Long Reef (LG), Myrmidon Reef (MYR), Rib Reef (RIB), Orpheus Island/Pelorus Island (OPI), Great Keppel Island (KEP), One Tree Island (OTI), Solitary Islands (SOL) and Lord Howe Island (LHI). Pie charts represent frequencies of the five genetic groups α-ϵ and the outgroups *P. verrucosa* and *P. meandrina* (n = 145; Sample size for each location indicated by pie size, see white pies on the left for size reference)

2.2

MATERIALS AND METHODS

2.2.1 Specimen collection and morphological identification

One hundred and forty-five colonies, falling within the accepted range of morphological variation described in *Pocillopora damicornis sensu stricto* (Veron & Pichon, 1976; Veron, 2000), were collected on SCUBA from 13 different reef slope sites along Eastern Australia (Fig. 2.2; Supplemental Material, ESM Table S1). Small fragments (approximately 1-2cm) of each colony were preserved in 90% ethanol for molecular analyses. Colonies were classified as one of five previously described *P. damicornis* morphotypes (here defined as individuals that are characterised by similar gross morphology) Veron & Pichon 1976; Veron 2000; Fig. 2.1; also see chapter 5 for more details of classification). Briefly, type α has a compact colony growth form with round branch endings and corresponds with morphotypes shown as colonies 60 and 61 in Veron & Pichon (1976) (see Fig. 2.1). Type β has fragile, elongate, terete branches, with pointed branch tips and corresponds to the types shown as 65–68 by Veron & Pichon (1976) (Fig. 2.1). In contrast to the others, both of these types show a mostly well-developed columella at the branch tips. Type γ has robust branches and correspond to those shown by Veron & Pichon (1976) as figures 62 and 63, respectively (Fig. 2.1). Type δ is found only in subtropical environments and grows in a distinct flat, almost plate-like colony form (Fig. 1 p. 72 in Veron 1986). Type ϵ is a compact, cespitose morphotype with short, thick and crowded branches (Fig. 2.1) corresponding to 58 and 59 by Veron & Pichon (1976). Examples of representative colonies for each morphological group are presented in Fig. S2.1.

2.2.2 DNA extraction, PCR amplification, sequencing and statistical haplotyping

Total DNA was extracted from all specimens using the DNeasy Extraction kit (Qiagen) according to the manufacturer's instructions and subsequently stored at -20°C.

Coral DNA amplification and sequencing: Two highly variable mitochondrial regions, known to be useful for species delineation in pocilloporids (an open reading frame (ORF) of yet unknown function and the putative control region (CR); (Flot & Tillier, 2007; Flot et al., 2008)), the commonly used nuclear Internal Transcribed Spacer (ITS2; Flot & Tillier 2006) and the nuclear gene Heat Shock Protein 70 (HSP70) were PCR-amplified and sequenced. Primers for HSP70 were developed for this study based on conserved regions in published HSP70 sequences of *P. damicornis* and *Stylophora pistillata* (Genbank Accession AF152004 and AB201749) using Primer3, v1.1.4 (http://frodo.wi.mit.edu/primer3/) (Rozen & Skaletsky, 2000); HSP70A-F, 5'-CCT GGT TCA ATC CGA CAG A-3', and HSP70A-R, 5-TGT TCA GCT GTT TTC CTT CG-3' amplify an 842bp fragment of the first half of the HSP70 gene (HSP70A) ; HSP70B-F, 5'-CTA TCC AGG CAG CGG TCT T-3', and HSP70B-R, 5'-TGG TGA ACA CAC TTG CTG TAG A-3' amplify a 698 bp fragment of the second half of the HSP70 gene (HSP70B). HSP70A and HSP70B regions were concatenated for further analyses. Although HSP represents a multi-copy gene family, HSP70 is known to be a single-copy gene in several invertebrates (Rieger et al., 2007). I am confident that only one HSP70 paralog has been amplified in this study, given the specificity of the oligos and the topological congruence with the ITS2 marker (see results section). All PCR reactions were performed in 30μl volumes containing 0.3μg 100xBSA, 2.5U HotStart iTaq DNA polymerase, 3μl 10x PCR buffer (20mM $MgCl_2$), 500pmol of each primer, 250μM of dNTPs (total), and approximately 40ng of template DNA. The thermal cycling protocol was 5min at 94°C followed by 30x (30s at 95°C; 30s at 57.5°C (CR) or 58.5°C(ORF) or 59°C (HSP70A and HSP70B) or 53°C (ITS2) and 1min at 72°C) and ended with a 10min extension at 72°C. PCR products were purified and sequenced in both directions for the mitochondrial markers and ITS2, and in one direction for the HSP70 markers (using HSP70A-F and HSP70B-R), by Macrogen Inc., Korea. Electropherograms were edited using Sequencher 4.9 (Gene Codes, Ann Abor, Michigan, USA) and manually aligned in BioEdit, v7.0.5.3 (Hall, 1999). Individuals with double peaks in their chromatograms were considered to be heterozygotes (Flot et al., 2006) and sequences were delineated using seqPHASE (Flot, 2010) when both alleles were of identical length and CHAMPURU (Flot, 2007) when alleles were of different length. The latter was only found in the ITS2 region. After delineation, heterozygote individuals were represented by their two allelic sequences in the alignments used for network construction.

Sequence analysis and genealogical network reconstruction Alignment statistics (number of haplotypes, polymorphic sites, parsimony informative sites, haplotype diversity, nucleotide diversity, average number of nucleotide differences) were evaluated in DnaSP v5 (Rozas et al., 2003) for each DNA region. All sequences generated in this study were deposited in GenBank accession numbers JX624790-JX625114, JX985584-JX985620. Network v4.5.1.6 (http://www.fluxus-technology.com) was used to examine genealogical relationships between morphotypes using the median-joining algorithm (Bandelt et al., 1999).

Coral genealogical network analysis: Median-joining networks were initially constructed based only on the ORF as this represented the largest and most comprehensive data set). Subsequently, four DNA regions were sequenced from 37 selected colonies, representing the five identified morphotypes. The morphologically distinct and closely related *Pocillopora verrucosa* (Ellis & Solander 1786) (Colony OPI 7), and *Pocillopora meandrina* Dana 1846, were included in both analyses for outgroup rooting. Furthermore, publicly available sequences from other pocilloporid species were included in our final data set for further genetic comparisons.

For each of the nuclear markers, haplowebs (i.e.

haplotype networks that incorporate additional connections among haplotypes that co-occur in heterozygote individuals) were computed to visualise single-locus fields for recombination (sl-FFRs). These sl-FFRs reflect groups of individuals that share a common allele pool and thus can be considered separate species following the criterion of mutual allelic exclusivity (Flot et al., 2010). The delineation of closely related or recently diverged species that do not yet display reciprocal monophyly is considered particularly effective when congruence in sl-FFRs obtained from multiple independent DNA markers is observed (Flot et al., 2010).

Symbiont genealogical network analysis: Median joining networks were computed from *Symbiodinium* ITS2 sequences produced in this study merged with ITS2 sequences of known *Symbiodinium* strains to identify *Symbiodinium* clades and assign them to East Australian *Pocillopora* mitochondrial lineages. The recovered clades were then scored on a binary matrix as presence/absence for each *Pocillopora* morphotype and the associations between host morphotypes and *Symbiodinium* clades were examined by means of principal coordinate analyses (PCA) in GenAlEx v6.4 (Peakall & Smouse, 2006).

2.2.3 Evaluating reproduction mode among selected types of *P. damicornis*

Colonies of types α and β were collected at Orpheus Island and maintained in a flow-through seawater system at the Australian Institute of Marine Science (AIMS, Townsville). Following larvae release, adults and planulae were genotyped at three polymorphic microsatellite loci (Pd3_005, Pd3_008 and Pd2_007; Starger et al. 2008) to determine the allelic contribution of the adults to the larval stages and assess whether sexual or asexual reproduction occurred (i.e., under asexual reproduction, planulae should only display alleles of maternal origin). DNA was extracted from over 100 planulae using a protocol optimised for single-fly DNA preparation for PCR (Gloor et al., 1993). To streamline processing, planulae from each colony were pooled into lots containing three planulae for DNA extraction and genotyping as asexual reproduction was expected (Ayre & Miller, 2004). To verify this method, I pooled DNA of multiple planulae of known and different microsatellite profile and the total DNA was genotyped *de novo* with the same microsatellite loci. All alleles from all planulae were always recovered in this process, suggesting that our system was not compromised by PCR artefacts All loci were multiplexed in a 10μl PCR reaction containing 5μl 2x Qiagen®Multiplex PCR Master Mix, 200pmol of each primer and approximately 40 ng template DNA. The thermal cycling protocol was 15min at 94°C followed by 30x (30s at 95°C; 1min at 60°C and 1min at 72°C) and ended with a 10min extension at 72°C. PCR products were purified using a Sephadex gel, genotyped on a MegaBACE 1000 capillary sequencer with an internal size standard (ET 550R) and scored using MegaBACE Genetic Profiler v. 1.2. Microsatellite diversity (number of alleles, observed heterozygosity, expected heterozygosity) across a sample of 25 adults (including natal colonies of larvae) from Orpheus Island was calculated in GenAlEx v6 (Peakall and Smouse 2006). Probabilities of identity by random sexual mating (Waits et al., 2001) were calculated using an AMOVA approach (Excoffier et al., 1992) to determine whether the corals were reproducing asexually or sexually.

2.2.4 Evaluating association between *Symbiodinium* clades & *P. damicornis* morphotypes

To investigate whether morphologically distinct groups were characterised by specific *Symbiodinium* clades, I used denaturing gradient gel electrophoresis (DGGE) and direct sequencing to identify the *Symbiodinium* clades found in 37 *Pocillopora* specimens representing a subsample of each of the five morphotypes following the method described by LaJeunesse (2002). Qiagen multiplex PCR reagents with HotStar Taq were used following the standard protocol provided by the manufacturer in a touchdown PCR protocol to amplify the ITS2 sequence marker using *Symbiodinium* specific primers (ITSintfor2 and ITS2clamp; LaJeunesse & Trench 2000). PCR products were run on a 30-65% denaturing gradient run at 65°C at 75V for 17 hours, diagnostic bands were excised (1-3 diagnostic bands were excised for each morphotype), re-amplified (using the same primers as above but without GC clamps) and sequenced in both direction by Macrogen Inc., Korea. Sequences were manually aligned in BioEdit (Hall, 1999).

2.3

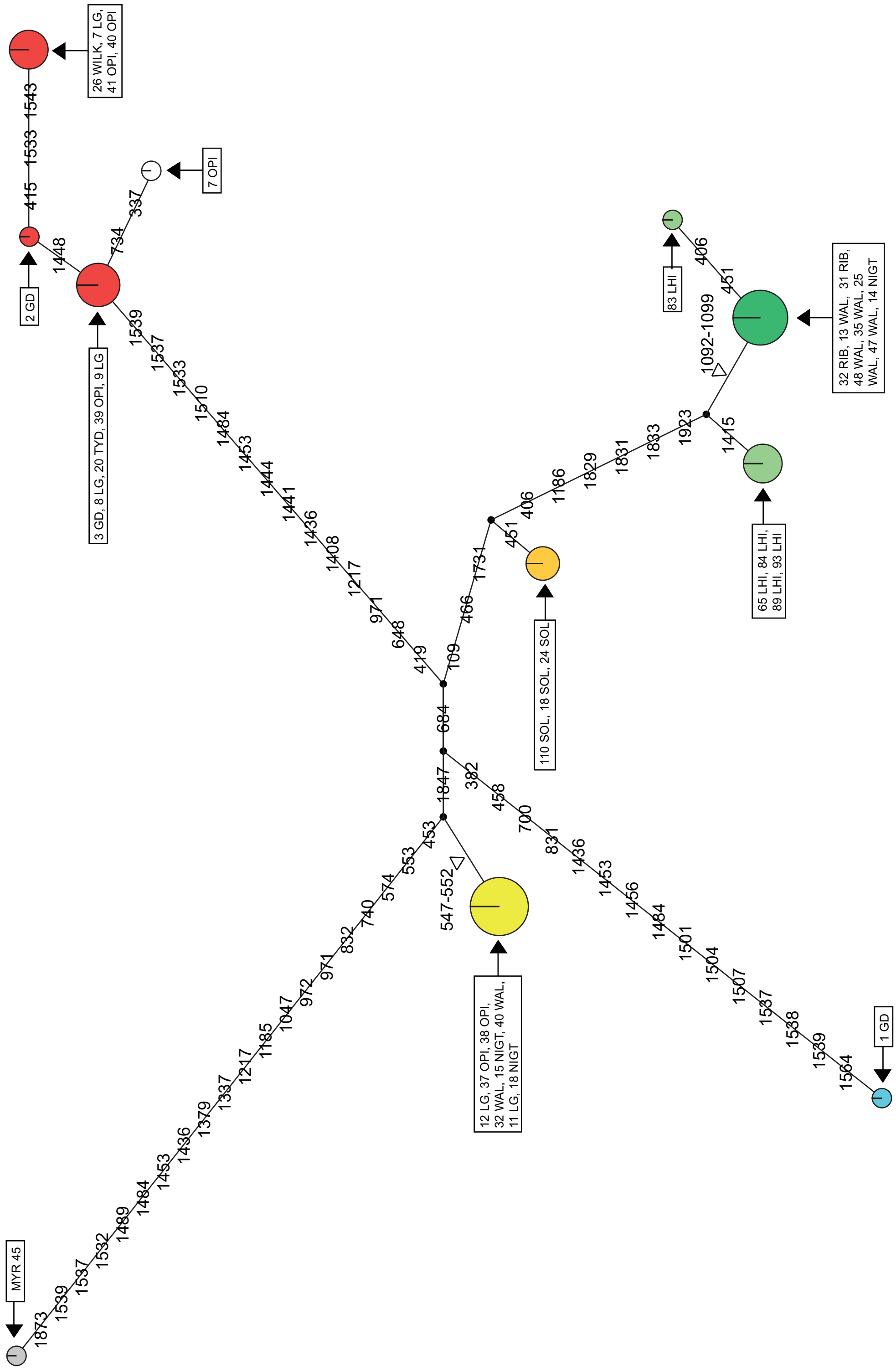
RESULTS

2.3.1 Sequence analysis & genealogical network reconstruction

Genealogical network reconstructions from 145 specimens of the mitochondrial ORF region revealed five genetically distinct lineages within *Pocillopora damicornis*, herein named types α, β, γ, δ and ϵ, separated by a minimum of six evolutionary steps (counting insertion/deletions as single character states; see Table 2.1 for alignment statistics). Combinations of these types are found in sympatry at reef slopes sampled along the GBR and can be clearly associated with the five previously recognised morphotypes of *P. damicornis* (Figs. 2.2, S2.2).

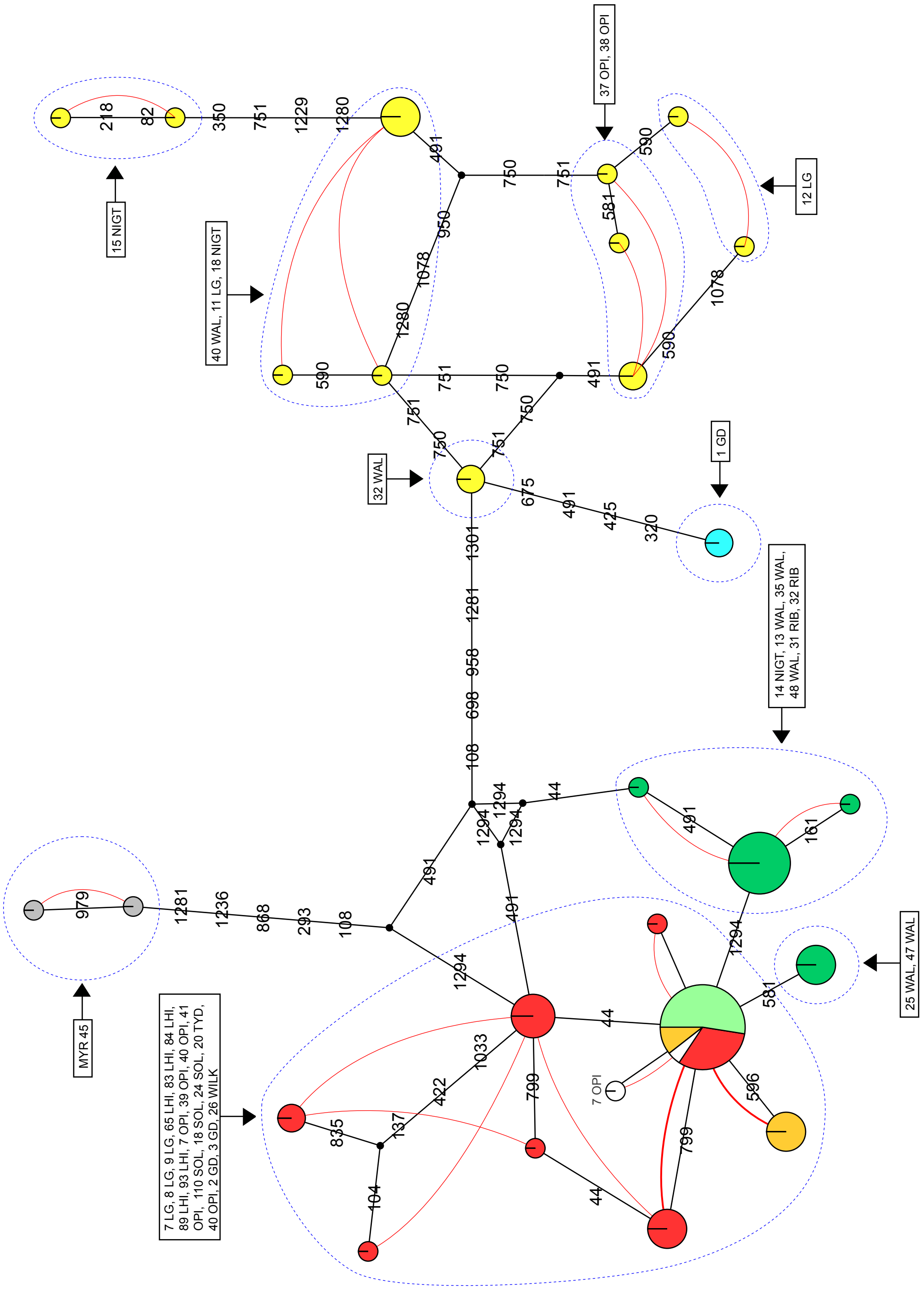
Haplotype networks inferred from CR sequences of a sub-set of the colonies (Fig. S2.3) and from selected concatenated CR and ORF sequences (Fig 2.3a) revealed eleven highly differentiated haplotypes and further supported the aforementioned findings (Figs. 2.3a, S2.3). An eight-bp deletion in the CR (bp 1091-1098) was found to be characteristic feature of type α from GBR (Figs. 2.3a, S2.3), although some type α specimens collected from Lord Howe Island (LHI) (further referred to as type α-LHI) lacked this deletion. A six-bp deletion (bp 546-551) in CR was found to be diagnostic of type β (Figs. 2.3a, S2.3). Interestingly, mitochondrial sequences of type γ formed a single cluster with sequences of the outgroup *P. verrucosa* (Fig 2.3a).

At global geographical scales, median joining networks of the ORF marker supported an extended distribution range of the *P. damicornis* mitochondrial lineages throughout the Pacific Ocean (Fig. 2.4). For example, the same type α ORF haplotype, previously reported as *P. damicornis*, was found in Taiwan (EU400213) and Hawaii (EU374277). Similarly, a type β haplotype was previously reported as *P. damicornis* type F from Kenya (FJ424157) and *P. damicornis* from Hawaii (EU374272). Whilst four of our *Pocillopora* morphotypes from Eastern Australia (α, β, γ and the outgroup *P. meandrina*) grouped with published sequences, types δ and ϵ were completely new. Interestingly, the colony sampled as the outgroup *P. verrucosa* fell into type γ. The latter seems closer to *P. molokensis* from Hawaii (EU374298), *P. damicornis* NF type from Kenya (FJ424172), *Pocillopora* sp. type 3a (HQ378760) and type 3b (HQ378761) from Panama than with other *P. damicornis* morphotypes from the GBR (Fig. 2.4). Contrary to the highly informative mitochondrial markers, the nuclear HSP70 region assigned types δ, γ and α -LHI into one sl-FFR in which the outgroup *P. verrucosa* was included (Fig. 2.3b). Types δ, γ, α and α-LHI on the other hand formed a single sl-FFR when the ITS2 region was analysed (Fig. 2.3c). High levels of genetic variation were recovered for type β from both HSP70 and ITS2 nuclear markers, and as expected, type β alleles were partitioned in more than one sl-FFR. Combining HSP70 and ITS2 sl-



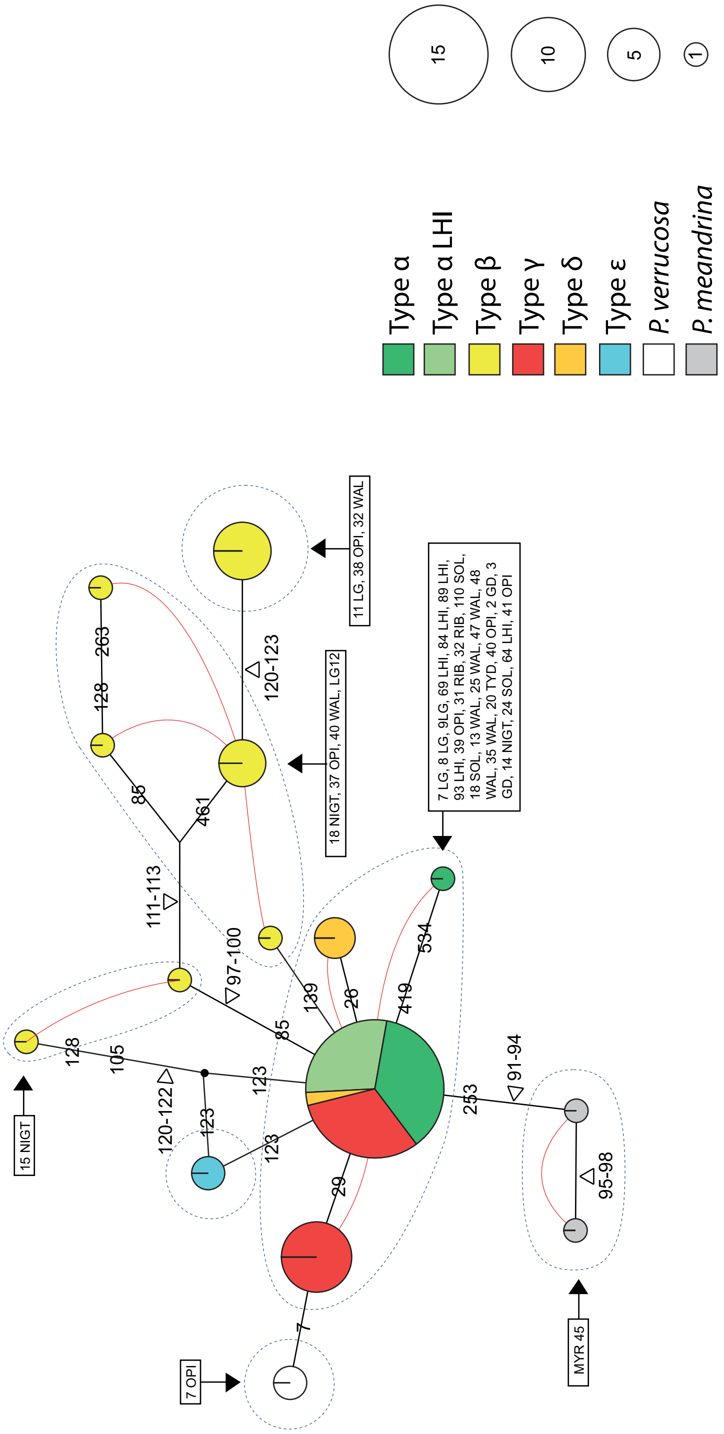
(a)

Figure 2.3: Haplotype networks. Mitochondrial regions (CR (1–1,265 bp) and ORF (1,267–2,106 bp)). (*continued*)



(b)

Figure 2.3: HSP70. (*continued*)



(c)

Figure 2.3: ITS2. Red lines connect haplotypes of heterozygote individuals. Blue circles indicate single-locus fields for recombination (sl-FFRs) characterised by mutual allelic exclusivity. Triangles indicate bp deletions/inserts. Abbreviations indicate sample locations (see Fig. 2.2)

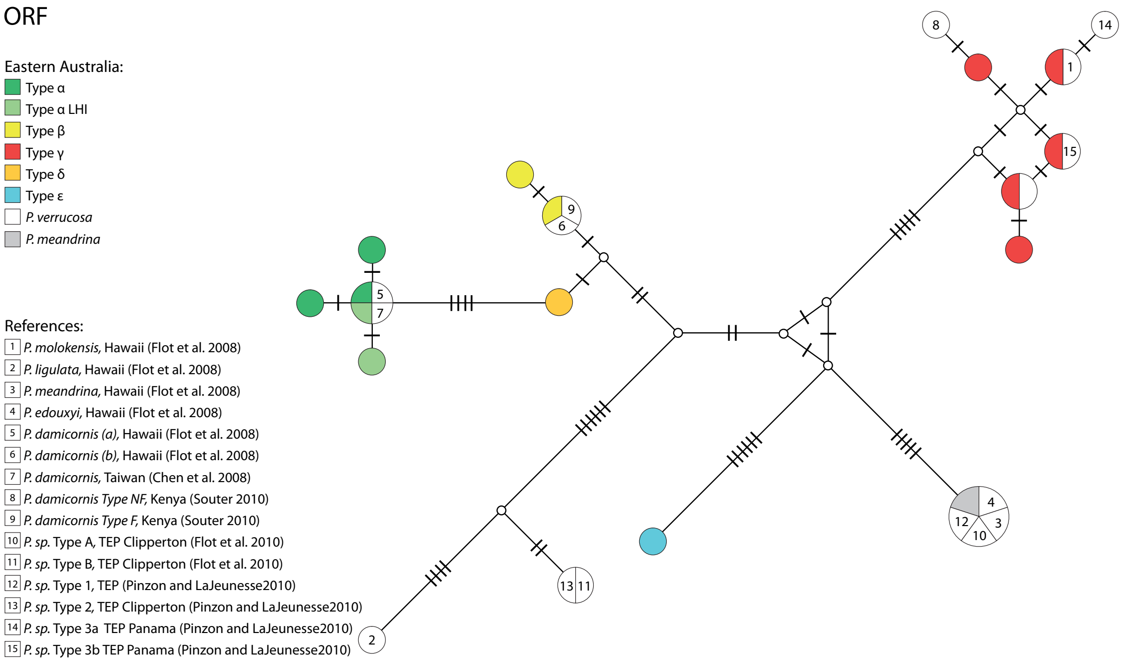


Figure 2.4: Haplotype network based on ORF DNA sequence data and incorporating published sequence data from other locations across the Indian and Pacific Oceans (total alignment length = 593 bp)

FFRs into a single ml-FFR analysis, four partitions corresponding to putative species boundaries between the outgroup *P. meandrina*, type β, type ϵ and a group comprising the remaining types (α, α-LHI, γ and δ) were recovered (Fig. 2.5).

2.3.2 Evaluating reproduction mode among selected types of *P. damicornis*

Multi-locus microsatellite genotypes obtained from all planulae were identical to their natal colonies for type α (n = 1 adult; 30 planulae released after the full moon in May 2010) and type β (n= 7 adults and 70 planulae; released after the new moon in October and December 2009, and January, April and May 2010). Allelic diversity was relatively high at all loci (Table S2.2) and the probability of identity very low ($p< 0.0001$), indicating that type α and type β colonies had reproduced asexually.

2.3.3 Evaluating association between *Symbiodinium* clades & *P. damicornis* morphotypes

Seven of the *Symbiodinium* types identified in this study were previously described, and six were novel (c, i, j, k, m, h; Fig. 2.6a). Interestingly, five of the clades (i, j, k, m and C100; described from LHI by Wicks et al. 2010) were found only in individuals from subtropical populations and may well be endemic to specific locations. For example, clades i, j and k were only found in the Solitary Islands and m was unique to Lord Howe Island. Based on sequence similarity, all novel types were attributed to *Symbiodinium* clade C except "h" that showed close similarity to *Symbiodinium* clade H5 (AM748609; 96% identity), a clade previously found in *Foraminifera* but never observed in corals until now. Highly similar *Symbiodinium* signatures were observed in the genetically distinct mitochondrial types β and γ (Fig. 2.6b). Although the nuclear regions failed

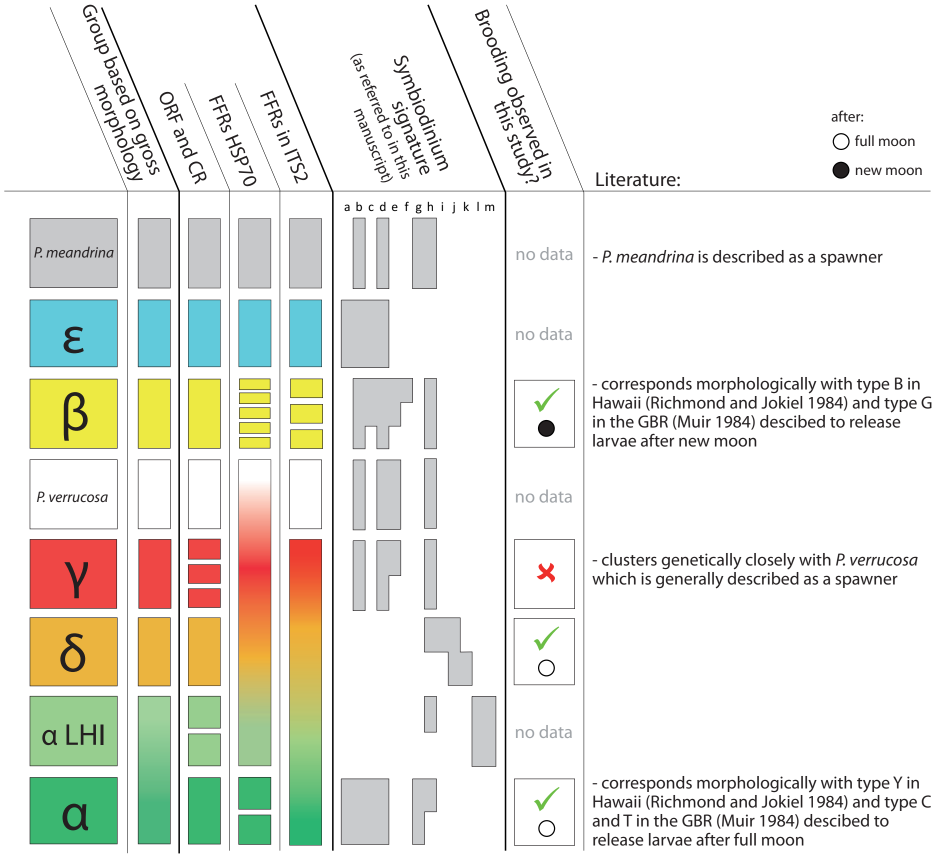


Figure 2.5: Summary of variation in morphology, genetics, symbiont signature and reproduction among each of the *Pocillopora* types. Differences and similarities for the assessed lines of evidence (columns) among the genetic lineages (rows, represented by the different colours) are indicated by rectangles.

to resolve *P. damicornis* types α and γ, consistent differences in the symbiont signature were observed (Figs. 2.3, 2.6). Of all morphotypes, *P. damicornis* type ϵ had the most similar symbiont signatures to type α as they shared 4 out of 5 *Symbiodinium* clade C types (Figs. 2.6, S2.4). Further, tropical and subtropical samples were clearly distinct.

2.4

Discussion

The scleractinian reef-building coral *Pocillopora damicornis* consists of multiple genetically distinct mitochondrial lineages, herein named types α, β, γ, δ and ϵ, characterised by reproductive and symbiont data peculiarities and corresponding to five previously reported *P. damicornis sensu lato* morphotypes (Figs. 2.1, S2.1). Only types β , ϵ and α, γ, δ, could be resolved by the ml-FFR analysis of the nuclear markers HSP70 and ITS2 and are therefore here conservatively interpreted as three biologically distinct species. The mitochondrial types reported here have been previously interpreted as extreme morphological

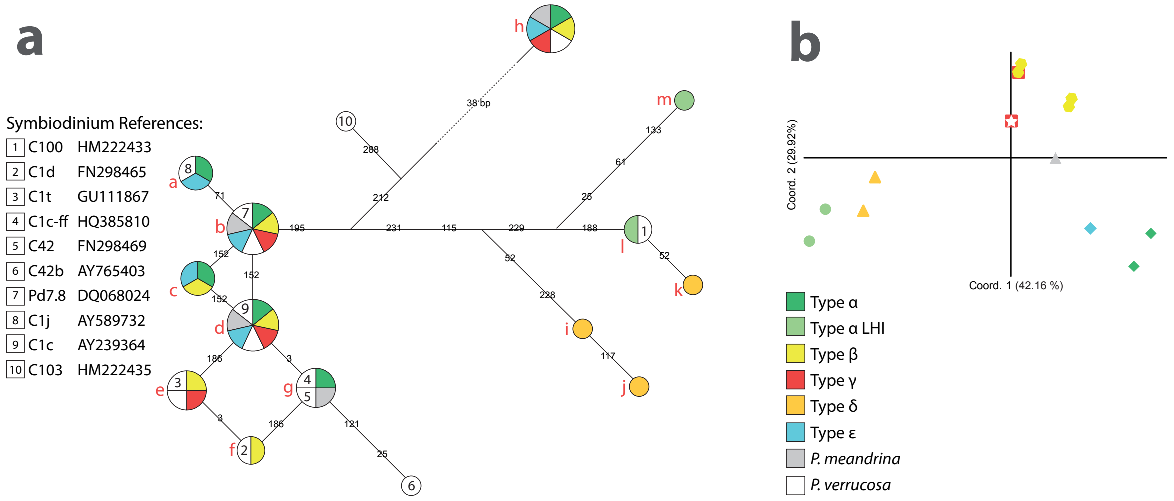


Figure 2.6: Genealogical relationships of symbiotic zooxanthellae (*Symbiodinium*) based on ITS2 types for each genetic lineage (represented by colours). GenBank reference sequences of closely related *Symbiodinium* clades are reported to the left; b: Principle Co-ordinates Analysis of *Symbiodinium* clades found within each of seven *Pocillopora* types from Eastern Australia .

variants of *P. damicornis sensu lato* (Fig. 2.1); they are often found in sympatry and they have been likely overlooked in earlier studies of the species in Eastern Australia (Figs. 2.2, S2.2). Assuming five mitochondrial lineages to be a single biological species may well explain a) the wide range of reproductive modes that has been attributed to *P. damicornis* across its reported distribution range (e.g., Richmond & Jokiel, 1984; Stoddart & Black, 1985; Ward, 1992; Tanner, 1996) the mismatch between life history and population structure reported previously for *P. damicornis* from the GBR (Ayre & Miller, 2004; Miller & Ayre, 2004; Sherman et al., 2006).

2.4.1 Phenotypic plasticity vs. genotypic variation in *P. damicornis*

Phenotypic plasticity is a trait often associated with corals and has often challenged classical taxonomy in several coral groups (e.g., Miller, 1994a; Shaish et al., 2007; Todd, 2008). In some taxa, transplant experiments have demonstrated that colony morphology can largely vary with habitat changes (e.g., Willis & Ayre, 1985; Bruno & Edmunds, 1997). The *P. damicornis sensu lato* morphological variants identified here are clearly associated with genetically distinct lineages, thus reinforcing the growing literature revealing cryptic speciation in a wide variety of coral groups and within so-called morphologically variable taxa, e.g., *Montipora* spp. (Stobart & Benzie, 1994); *Montastrea annularis* (Medina et al., 1999); species within the Faviidae (Nunes et al., 2008); species within the Poritidae (Forsman et al., 2009) and species within the Pocilloporidae (Flot et al., 2011). It should be noted, however, that while our results demonstrate that accepted ecomorphs of *P. damicornis* may represent different species, I do not completely discount the influence of eco-phenotypic variation in pocilloporid diversity and systematics. I acknowledge that the extents of morphological plasticity are variable and may partially overlap between two mitochondrial types (e.g., type β colonies sampled ranged from having a thin, elongate colony shape to a more stunted branching one). Further studies are therefore necessary to reveal the importance of environmental influence and measure the colonys transcriptomic response both responsible for the

Table 2.1: Sequence and alignment statistics for mitochondrial and nuclear sequences of *Pocillopora* spp. for core data set (n=37). Size of alignment (bp), number of haplotytpes (H), number polymorphic sites (N_{ps}) number parsimony informative sites (N_{pi}), haplotype diversity (H_D), nucleotide diversity (P_i), average number of nucleotide differences (k).

	bp	H	N_{PS}	N_{PI}	H_D	P_i	k
CR	1266	9	25	11	0.823	0.0042	5.246
ORF	840	9	30	19	0.859	0.0104	8.429
HSP70	1329	27	36	28	0.906	0.0041	5.433
ITS2	549	12	12	8	0.704	0.0029	1.561

range of morphological variation revealed by each of the mitochondrial types.

2.4.2 Reviewing reproductive variation among types of *P. damicornis*

Distinct modes and timing of reproduction have been reported for *P. damicornis* in the past and have often been interpreted as population- or geographic location-specific (Stoddart & Black, 1985). For instance, Tanner (1996) described larvae release after full moon in summer for the southern GBR, which is in agreement with our observations from One Tree Island (23 November - 03 December 2010) where only type α colonies were identified. Muir (1984) described two cycles of planulation in *P. damicornis* morphotypes from the central GBR, namely, types C and T which released planulae after the full moon and type G which planulated after new moon, in accordance with our types α and β (based on morphological similarities and observed planulation data). Furthermore, Richmond & Jokiel (1984) described a "yellow" morphotype corresponding to Muir's (1984) type G and a brown morphotype in Hawaii, the latter with different timing of reproduction, morphologically corresponding to Muir's (1984) types C and T. The presence of both mitochondrial types α and β in Hawaii (Fig. 2.4), their sympatric distribution and similar reproductive strategy in the two locations (GBR and Hawaii) suggests that the "brown" and "yellow" morphs likely correspond to types α and β recovered in this study. Both types α and β appeared to reproduce via asexually brooded planulae. Although our findings are in agreement with previous studies of *P. damicornis* from the GBR (Ayre & Miller, 2004; Sherman et al., 2006), I might have missed rare sexual brooding as our sample sizes were small and sexually produced planulae have been reported in *P. damicornis* from Taiwan (Yeoh & Dai, 2010).

Type γ colonies were not observed to release planulae and it is possible that they broadcast spawn gametes. This is plausible as morphological and genetic similarities indicate that *P. damicornis* type γ is either identical or closely related to *Pocillopora verrucosa* (Figs. 2.3, 2.4). The latter has been described as a broadcast-spawning, simultaneous hermaphrodite from South Africa (Kruger & Schleyer, 1998) and the Maldive Islands (Sier & Olive, 1994). Interestingly, the species has been reported as a brooder at Enewetak Atoll (Stimson, 1978) and the Philippines (Villanueva et al., 2008), and to release planulae simultaneously with *P. damicornis* after new moon. Population genetic profiles in *P. verrucosa* resemble patterns of a sexually reproducing species (Ridgway et al., 2001, 2008) similar to that reported for *P. damicornis* type NF by Souter (2010). The latter type together with types 3a and b identified by Pinzón & LaJeunesse (2010) in Panama, were recovered within our type γ and *P. verrucosa* (Fig. 2.4) and may well explain why tropical eastern pacific (TEP) *P. damicornis* is reported to be a broadcast spawner (Glynn et al., 1991) and to show sexual population structure (Combosch & Vollmer, 2011). In addition, Souter

(2010) reported clonally propagated populations for *P. damicornis* (type F in Souter 2010), which is closely related to type β in our study (Fig. 2.4) and seems to be an asexual brooder. The aforementioned observations suggest that reproductive differences are lineage- rather than location-specific. In light of the types sympatric distribution, it cannot therefore be excluded that *P. damicornis* and *P. verrucosa* have been confused in previous studies.

2.4.3 *Pocillopora* types vs. *Symbiodinium* signatures

Species-specific symbiont associations have been recently identified in the TEP for three species of *Pocillopora* (Pinzón & LaJeunesse, 2010). In addition, in *Seriatopora hystrix* concordant genetic partitioning of coral host and its symbionts was found to be associated with different habitats along the GBR (Bongaerts et al., 2010). Maternal transmission of symbionts in *P. damicornis* and *P. verrucosa* (Richmond & Jokiel, 1984; Sier & Olive, 1994) may well explain the correlation of mitochondrial host DNA and *Symbiodinium* signature. *Symbiodinium* type C1 has been reported in *P. damicornis* (e.g., (e.g., LaJeunesse et al., 2004; Magalon et al., 2007; Sampayo et al., 2007) and in this study a limited number of lineage-specific associations between combinations of *Symbiodinium* type C1 sub-clades and *Pocillopora damicornis* types has been identified (Fig. 2.6). In addition to the previously described *Symbiodinium* C100 clade in *P. damicornis* of LHI (Wicks et al., 2010), I also report a novel type at Lord Howe Island in this study (referred to as m, Fig. 2.6a). Interestingly, none of the types found at LHI were shared by *Pocillopora* of the Solitary Islands (Fig. 2.6a). The novel *Symbiodinium* clade (referred to as h, Fig. 2.6a) sporadically found in our samples seems to be closely related to *Symbiodinium* type H which has never been described in corals.

2.4.4 *Pocillopora* in a global perspective and cryptic species boundaries

Multiple genetically distinct lineages have been recently acknowledged in the Indo-Pacific within *P. damicornis* (Flot et al., 2008; Pinzón & LaJeunesse, 2010; Souter, 2010). The combination of sequence data from Flot et al. (2008) and Souter (2010) with sequences produced in this study confirms the existence of eight genealogically distinct mitochondrial *Pocillopora* lineages from the Indian and Pacific Oceans (Fig. 2.4). Of these, six occur at times in sympatry along Eastern Australia, five belong to *P. damicornis sensu lato*, two (types δ and ϵ firstly reported in this study) are novel (Fig. 2.4). The aforementioned lineages are characterised by a relatively low number of synapomorphic characters, yet the degree of differentiation is considered characteristic for scleractinians especially as the coral mitochondrial genome evolves much slower than in other metazoans (e.g., Shearer et al., 2002; Hellberg, 2006). Mitochondrial ORF sequences of *Pocillopora molokensis* and *P. verrucosa* are clearly associated with our *P. damicornis* type γ; the association between the latter and *P. verrucosa* is further supported by similarities in gross morphology and symbiont signatures. I therefore assume that the assemblage of type γ, *P. verrucosa* and *P. molokensis* suggests the same taxonomic unit, which has been erroneously misidentified in previous studies (Pinzón & LaJeunesse, 2010; Souter, 2010).

The multiple approaches used to reveal species boundaries within the *P. damicornis* species complex provided variable levels of resolution. For instance, types β and ϵ were supported by all means as distinct valid taxa (Fig. 2.5). Types α, γ and δ on the other hand, although clearly distinct by means of mitochondrial markers, *Symbiodinium* signatures and gross morphology, could not be resolved by the nuclear markers. Several explanations may account for this incongruence: first, the here employed nuclear regions may suffer from ancestral, unresolved polymorphisms. This hypothesis seems implausible in our case since lineage sorting should be faster especially for the isolated subtropical populations. Second, the HSP70 locus could be under positive selection and therefore little variation could be recovered among types. However, the neutrally evolving ITS2 marker reproduced the same results as HSP70 (Fig. 2.3). I therefore attribute the lack of divergence among types α, γ and δ on instances of occasional introgressive hybridisation among the mitochondrial lineages. Likewise, hybrids between *P. damicornis* and *Stylophora pistillata* were found on LHI (Miller & Ayre, 2004) and hybridisation may occur regularly in a range of coral species (see Willis et al., 2006).

Hybridisation is expected to occur occasionally in *P. damicornis sensu lato* because differences in reproductive mode and timing occur only among some of the types (Fig. 2.5). In fact, a mixed mode of reproduction has been described for unknown *P. damicornis* lineages in some locations (Stoddart & Black, 1985; Ward, 1992). In similar cases, a species concept based on strict reproductive isolation or distinct phylogenies cannot be assumed despite our data implying that certain characteristics (reproduction, morphology and symbionts association) are lineage-specific. A combination of morphological and molecular approaches has successfully revealed cryptic species in *Stylophora* species (Flot et al., 2011; Stefani et al., 2011), yet the degree of diversification encountered in *P. damicornis* may be very common in other so-called widespread corals. Alternative species concepts, such as the Unified Species Concept (De Queiroz, 2007) may therefore be necessary in the future to provide support for robust taxonomic units on the basis of multiple lines of evidence such as multiple molecular marker systems, ecological, reproductive knowledge and morphological data.

CHAPTER
THREE

REPRODUCTION WITHIN *POCILLOPORA* SPECIES IN EASTERN AUSTRALIA

3.1
INTRODUCTION

Much conjecture exists about the reproductive biology of the coral genus *Pocillopora* despite it representing one of the most abundant and widely studied taxa of scleractinian corals. The genus is one of the few to include species that brood larvae (e.g. *P. damicornis*) and species that broadcast spawn gametes (e.g. *P. eydouxi*; Baird et al., 2009; Harrison, 2011). Spawning in corals refers to the release of gametes into the water column for external fertilisation and larval development, whereas brooding refers to the development of planula larvae within the polyps (Harrison & Wallace, 1990). Brooded planulae may originate from internal fertilisation of eggs or from parthenogenesis (Harrison, 2011). The ecological and evolutionary consequences of such a diversity of reproductive modes within a single coral genus has been the subject of considerable research over the last decades (e.g., Stoddart, 1983; Ward, 1992; Ayre & Miller, 2004; Sherman et al., 2006), although there still remain many gaps in our knowledge about when, and how, most *Pocillopora* spp. reproduce.

Pocillopora damicornis is thought to brood throughout most of its range (Table 3.1), and in Western Australia, Eastern Australia and Taiwan, molecular analysis indicate that brooded larvae are produced largely asexually (Stoddart, 1983; Ayre & Miller, 2004; Sherman et al., 2006; Yeoh & Dai, 2010). Other earlier examples of *Pocillopora* spp. brooding larvae have been discredited (Harrison & Wallace, 1990), except for one recent observation in the Philippines (*P. verrucosa*; Villanueva et al. 2008). In summary, of the seventeen formally accepted species of *Pocillopora* (Veron, 2000), three (*P. eydouxi, P. meandrina, P. elegans*) are broadcast spawners and two (*P. verrucosa* and *P. damicornis*) have a different mode of larval development among regions. In addition, some *P. damicornis* reproduce by brooding larvae and spawning gametes (Table 3.1).

At least some of the controversy around spatial variation in the reproductive mode of *Pocillopora* spp. is likely to be linked to the existence of cryptic species. For example, *P. damicornis* is now recognised to be a species complex rather than a single morphologically plastic species (Chap. 2). Of the five putative species within the *P. damicornis* complex, three were observed brooding (and at least two brood asexual larvae; Table 3.1). Spawning has been reported for *P.* cf. *damicornis* in the Eastern-Pacific (Glynn et al., 1991), although evidence shows that this species actually resolves genetically within one clade with

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P. verrucosa and *P. damicornis* Type γ and thus is genetically distant to species observed brooding in Australia (Chap. 2). Clearly, the difficulties in distinguishing even among what are considered morphologically distinct species of *Pocillopora* has contributed to the conflicting reports on reproductive behaviour within species.

The mode of reproduction in *P. damicornis* is also a matter of conjecture. Like many species, spawning has never been observed in *P. damicornis* rather, it has been inferred from the disappearance of gametes in histological samples (Muir, 1984; Glynn et al., 1991; Ward, 1992). In Australia, as in most other areas, all planula larvae examined appear to have been produced asexually (Chap. 2, Stoddart 1983; Ayre & Miller 2004; Sherman et al. 2006), however, the population genetic structure reflects random sexual reproduction with high genotypic diversity (e.g., Miller & Ayre, 2004; Sherman et al., 2006) suggesting important aspects of the life history of *P. damicornis* remain unknown. Here, we report the first observation of broadcast spawning of gametes in four *Pocillopora* species, including *P. damicornis*, and suggest that sexual reproduction is likely to occur regularly in pocilloporids on the GBR.

3.2

MATERIAL AND METHODS

3.2.1 Spawning observations

Colonies of four *Pocillopora* species were collected 1-2 days before full moon and maintained in a flow through seawater aquarium system at One Tree Island Research Station (23° 30‘29S; 152° 4‘37E) (*P. damicornis*) and Lizard Island Research Station (*P. eydouxi, P. verrucosa* and *P. meandrina*) (14° 41‘58S; 145° 26‘54E) in the summer of 2011/2012. The flow through water was turned off around midnight every night for up to 20 days following full moon to enable spawning to be observed. In addition to specimens observed to spawn (a total of ten colonies; Table 3.2), at Lizard Island two colonies of *P. damicornis* Type α (*sensu* Chap. 2) and two specimens of *P. verrucosa* were isolated, but did not spawn.

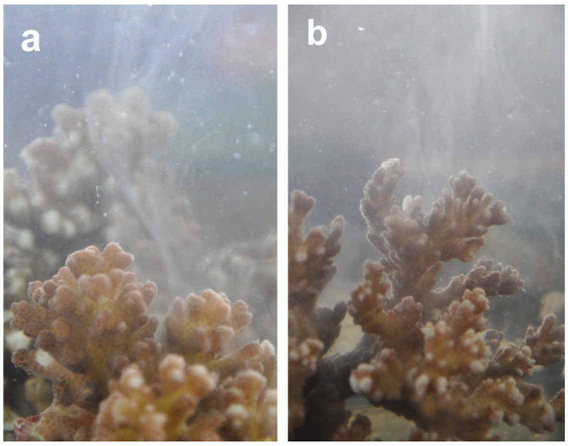

Figure 3.1: Sperm release by *Pocillopora damicornis*, One Tree Island

3.2.2 Specimen identification and phylogenetic analysis

Specimens were visually identified and categorised according to Veron (2000) and Chap. 2. For a subset of specimens belonging to each morphotype, identification was further verified by sequencing of the mitochondrial ORF region (Flot & Tillier, 2007) following protocols described in Chap. 2. Furthermore, differences in the population ecology of *P. damicornis* from Western Australia (population structure predominantly asexual; Stoddart 1984a; Stoddart 1984b) and *P. damicornis* in Eastern Australia (population structure predominantly sexual; Benzie et al. 1995; Ayre & Hughes 2000; Ayre & Miller 2004; Miller & Ayre 2004; Sherman et al. 2006 suggest these might be different species. Consequently, six specimens of *P. damicornis* from Rottnest Island, WA, were genotyped and sequences compared to existing data from the GBR. The alignment consisted of 11 sequences and 590bp

Table 3.1: Reproductive mode of *Pocillopora* species (∗inferred from histology).

Species	Location	Mode	Reference
P. verrucosa	Red Sea	Spawn∗	Fadlallah 1983; Shlesinger & Loya 1985; Séré et al. 2010
	Maldives	Spawn	Sier & Olive 1994
	Okinawa	Spawn	*in situ* Kinzie III 1993; Hirose et al. 2000
	Philippines	Brood	Villanueva et al. 2008
	Red Sea	Spawn	*in situ* and *ex situ* Bouwmeester et al. 2011
P. meandrina	Hawaii	Spawn	Riddle 2008, *in situ*; Riddle & Peck 2009, *in situ*
	Enewetak (as *P. elegans*)	Brood	Stimson 1978
P. eydouxi	Okinawa	Spawn	Kinzie III 1993, *ex situ*
	Hawaii	Spawn	Riddle & Peck 2009, *in situ*
P. damicornis	Western Australia	Brood	Stoddart 1983 (asexual); Stoddart & Black 1985
	Western Australia	Brood and spawn∗	Ward 1992
	Eastern Australia	Brood and spawn∗	Muir 1984 (spawning suggested based on the disappearance of eggs in histological samples)
	Eastern Pacific	Spawn∗	Glynn et al. 1991; Rodríguez-Troncoso et al. 2011
	Eastern Australia	Brood	e.g., Marshall & Stephenson 1933; Harriott 1983a; Ayre & Miller 2004 (asexual); Sherman et al. 2006 (asexual); *P. damicornis* Type α (asexual), Type β (asexual) and Type δ, Chap. 2
	Thailand	Brood	Kuanui et al. 2008
	Taiwan	Brood	Yeoh & Dai 2010 (sexual and asexual)
	Hawaii, Enewetak	Brood	Richmond & Jokiel 1984
P. elegans	Eastern Pacific	Spawn∗	Glynn et al. 1991

(NCBI accession numbers: JX983175-JX983186); reference sequences of previously identified cryptic species (Chap. 2) were additionally included in the analysis to identify and illustrate the genealogical relationships amongst the taxa investigated in this study (NCBI accession numbers: JX985589; JX985612; JX985610; JX985592; JX985613; JX985605). Phylogenetic hypotheses were generated in MEGA4 (Tamura et al., 2007) using the Neighbor-Joining algorithm under the JC correction and 100.000 bootstrap pseudoreplicated for nodal support (Felsenstein, 1985; Saitou & Nei, 1987).

3.3

RESULTS AND DISCUSSION

3.3.1 Spawning observations

During the southern hemisphere summer of 2011/12 we observed gamete release in *Pocillopora eydouxi, P. verrucosa* and *P. meandrina* at Lizard Island, and *P. damicornis* at One Tree Island (Table 3.1); two locations at opposite ends of the GBR. Genotyping of 590bp of the mitochondrial ORF region confirmed identifications based on morphology, except for *P. meandrina* and *P. eydouxi*, which share the same mitochondrial lineage (Flot et al., 2008), and therefore can not be distinguished by this marker. Spawning in all species occurred 1-2days following the full moon, approximately 45min after sunrise and continued for 2-3hrs. Unlike most broadcast spawning coral species, *Pocillopora* gametes were free-spawned separately, rather than packaged in egg-sperm bundles. Sperm release was evident as a dense cloud surrounding the colony (Fig. 3.1 & 3.2). Due to the small size (see below), eggs were difficult to see, explaining why this behaviour may have been missed previously (e.g., Ward, 1992; Séré et al., 2010). The eggs were negatively buoyant, approximately 80μm in diameter, and could easily be collected by siphoning the bottom of the aquaria below the colony. Eggs of *P. meandrina* (Fig. 3.3; ESM supplementary movie) and *P. eydouxi* contained algal symbionts, *Symbiodinium*. Ethanol preserved sperm samples of *P. damicornis* from One Tree Island also contained eggs. These eggs were 50-60μm, which matches the size of mature *P. damicornis* eggs in histological sections (Muir, 1984; Ward, 1992) (ESM Fig. S3.1). This strongly suggests the spawned eggs were mature rather than immature oocytes released due to handling. Fertilisation trials are required to confirm this unequivocally. Nevertheless, the release of sperm and mature eggs concurrently strongly suggests that sexual reproduction will occur. Numerous studies on sexual reproduction in other scleractinian corals (e.g., Babcock et al., 1986; Miller & Babcock, 1997; Willis et al., 1997) have demonstrated that spawning behaviour in the laboratory is identical to that in the wild. Thus broadcast spawning of gametes with external fertilisation and larval development is likely to be the spawning behaviour in the field and the source of the sexual recruits of *P. damicornis* reported by previous studies (e.g., Ayre & Miller, 2004).

Our observations are in agreement with reports from Hawaii (Fiene-Severns, 1998; Riddle, 2008; Riddle & Peck, 2009), Japan (Kinzie III, 1993) and the Red Sea (Bouwmeester et al., 2011) regarding time and mode of larval development in these *Pocillopora* species, suggesting daytime spawning with a lunar periodicity may be characteristic for this genus across its range. Importantly, for *P. damicornis* this is the first direct observation of gamete release. In addition, brooded planulae were released the night before gamete release, indicating that both reproductive strategies occur simultaneously in the same colony supporting the inferences of previous histological studies (Muir, 1984; Ward, 1992). Other coral species are known to vary their mode of reproduction in different geographic regions; however, *Goniastrea aspera* is the only other species in which individual colonies both brood and spawn (Sakai, 1997).

Figure 3.2: *Pocillopora meandrina* at Trimodal Reef, Lizard Island

3.3.2 Reviewing the populations structure of *P. damicornis* in the light of a mixed mode of reproduction

Histological studies have suggested that *P. damicornis* in Western Australia both spawns and broods (at Rottnest Island: Ward 1992) and that the brooded larvae are generated asexually (4). Sequence data confirmed that Western Australian specimens are genetically identical to *P. damicornis* Type α (Chap. 2) on the east coast of Australia (Fig. 3.4). Our observation on the GBR

Table 3.2: Summary of spawning observations presented

Dates of observation	Full moon	Location	Species (n)	Observation	Start (hrs)	Sunrise
12-13 Oct 2011	12 Oct 2011	One Tree Island	*P. damicornis* Type α (1)	Sperm released (*ex situ*) Fig. 3.1 Brooded planulae (over night)	06:10	05:23/ 05:22
11-12 Nov 2011	11 Nov 2011	Lizard Island	*P. meandrina* (1)	Spawn (*in situ*)	06:25	05:41
			P. meandrina (2)	Spawn (*ex situ*)		
			P. eydouxi (2)	Spawn (*ex situ*)		
			P. verrucosa (1)	Sperm released (*ex situ*) Fig. 3.2		
9 Feb 2012	8 Feb 2012	One Tree Island	*P. damicornis* Type α (2)	Spawning (*ex situ*)	approx. 06:00-07:00	05:35

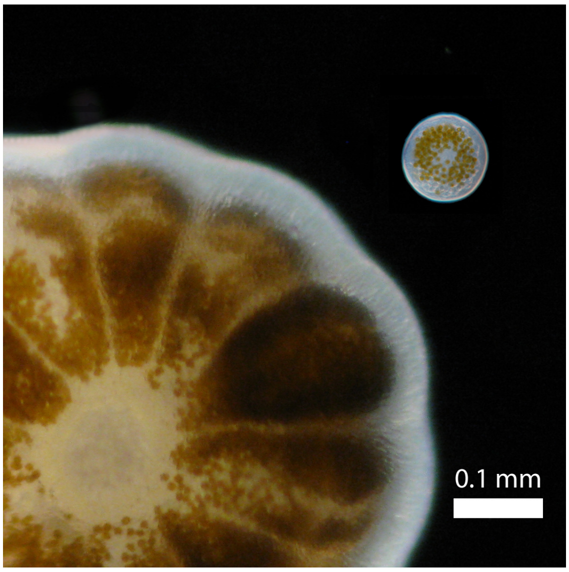

Figure 3.3: Brooded planula (*Pocillopora damicornis*, left) next to a spawned egg (*Pocillopora meandrina*, top right), indicating the size difference in *Pocillopora* between brooded and spawned offspring.

of spawning in a lineage of *P. damicornis* known to brood, the release of brooded larvae and spawning over consecutive nights in the same colony, as well as the overwhelming evidence that brooded planulae are generated asexually (Chap. 2, Ayre & Miller 2004; Sherman et al. 2006) suggests the same is true of *P. damicornis* on the east coast of Australia. Consequently brooding lineages within *P. damicornis* throughout Australia most likely have a mixed mode of reproduction (Fig. 3.4). Indeed, the clonal generation of planulae seems to be characteristic of these lineages within *Pocillopora* (Chap. 2) (Fig. 3.4), which contrasts with the sexual brooding reported in the sister genera *Stylophora* and *Seriatopora* (Ayre & Resing, 1986; Sherman, 2008; Douek et al., 2012).

The evolutionary advantages of a mixed mode of asexual brooding and sexual spawning are still poorly understood. While settlement behaviour and competency periods of brooded larvae of *P. damicornis* lineages are well studied (e.g., Richmond, 1987; Harii et al., 2002; Cumbo et al., 2012), nothing is known of the larval biology of spawned larvae in *Pocillopora*. Therefore, our observations represent an important foundation for future studies to further elucidate the differences between these larval types and the selective advantages of each mode of reproduction.

3.3.3 Future implications of observations

Typically for species with mixed modes of reproduction, asexual reproduction contributes to maintenance of local populations, with sexual progeny used for dispersal and recruitment to distant areas (i.e. the strawberry-coral model of Williams (1975)). However, population genetic studies of *P. damicornis* on the GBR (Benzie et al., 1995; Ayre & Hughes, 2000; Ayre & Miller,

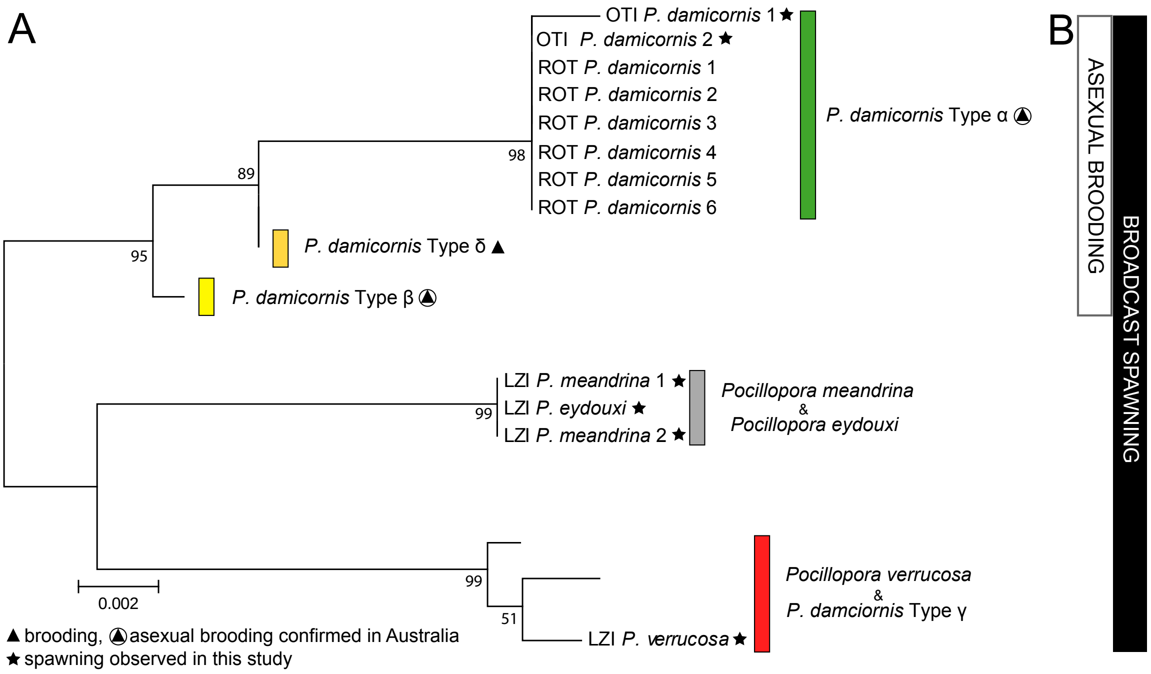

Figure 3.4: Mitochondrial phylogeny of *Pocillopora* specimens based on the ORF region. Colored bars denote genetically distinct lineages or cryptic species identified in Chap. 2. *Pocillopora eydouxi* and *Pocillopora meandrina* shared identical mitochondrial haplotypes whilst *Pocillopora verrucosa* was recovered within the same clade with *P. damicornis* Type γ. *Pocillopora damicornis* Type δ and Type β were added in the phylogeny to indicate the close genetic relationship of brooding species within the genus *Pocillopora*. Black and white vertical bars indicate the proposed reproductive strategies of these taxa in Australia. Sample locations, indicated by three letter codes, are as follows: OTI = One Tree Island; ROT = Rottnest Island; LZI = Lizard Island. Numbers represent bootstrap values.

2004; Sherman et al., 2006) show only limited evidence of local recruitment of asexual planulae, but genetic subdivision even on relatively small spatial scales among populations suggests dispersal of sexual larvae is also limited. Thus there is little evidence that *P. damicornis* conforms to the predictions of the strawberry-coral model. It may be that the opposite occurs within *P. damicornis*, with the sexual progeny from broadcast spawning settling locally (as occurs for other broadcast spawning species - Ayre & Hughes 2000; Miller & Ayre 2008a; Combosch & Vollmer 2011; Paz-García et al. 2012) and the larger, and potentially better provisioned asexual larvae being more widely dispersed. Indeed, Richmond (1987) reported that some brooded larvae of *P. damicornis* remained competent for over 100 days suggesting widespread dispersal of brooded larvae is possible. To date, population genetic studies have shown only limited evidence that asexual larvae of *P. damicornis* could be more widely dispersed, i.e. Ayre & Miller (2004) found colonies with identical genotypes on opposite sides of One Tree Reef. If these corals represent recruitment from asexual planulae, then dispersal on the scale of kilometres may well occur.

Clearly further research is required to tease apart the roles of the two larval types in *P. damicornis* and their dispersal potential. Striking differences in size of the asexual (~1000 μm) and sexual (~80 μm) (Fig. 3.3) larvae suggest dispersal potential may well vary between them, although both types do contain zooxanthellae and therefore have the potential to be autotrophic (Baird et al., 2009). Furthermore, the size difference raises questions of skeletal differences in early settlement between brooded and spawned larvae. While the skeletons of recruits of brooded offspring in this family are well studied (Baird & Babcock, 2000) and often a focus of recruitment studies (e.g., Schmidt-Roach et al., 2008), the small size of spawned larvae may result in observable differences in size between sexual and asexual recruits and thus may enable the brooded and spawned recruits to be distinguished at settlement, similar to recruits in *Porites* spp. (Babcock et al., 2003). The predictable and consecutive spawning over several months that we report here makes *Pocillopora* ideal for future experiments to address such questions, as well as aspects of both the ecological and evolutionary processes in this important group of corals, including the maintenance of mixed mode of reproduction and hybridisation in the genus *Pocillopora* (Chap. 2, Combosch et al. 2008; Flot et al. 2008).

Chapter 4 has been removed for copyright or proprietary reasons.

Schmidt-Roach, S; Miller, K. J.; Andreakis, N., (2013) Pocillopora aliciae: a new species of scleractinian coral (Scleractinia, Pocilloporidae) from subtropical Eastern Australia, Zootaxa, 3626 (4), 576-582

CHAPTER
FIVE

WITH EYES WIDE OPEN: A REVISION OF SPECIES WITHIN AND CLOSELY RELATED TO THE *POCILLOPORA DAMICORNIS* SPECIES COMPLEX (SCLERACTINIA; POCILLOPORIDAE).

5.1 Introduction

In recent years, molecular systematics has frequently challenged the morphology based taxonomy of scleractinian corals, and has significantly contributed to the understanding of coral phylogenetics and evolution (e.g. Chen et al., 1995; Fukami et al., 2004, 2008; Benzoni et al., 2010; Huang et al., 2011; Benzoni et al., 2012; Budd et al., 2012). In multiple cases however, a combination of genetic evidence with skeleton morphology, has resulted in a powerful approach for a comprehensive revision and delineation of coral species. For example, within the common scleractinian coral genus *Pocillopora*, phylogenetic inference and morphological data have revealed evidence of cryptic speciation (Flot et al., 2008; Souter, 2010; Schmidt-Roach et al., 2012a, 2013). However, some uncertainty remains in the link between morphological and molecular species boundaries in this genus (Pinzón & LaJeunesse, 2010; Pinzón et al., 2013).

In *Pocillopora*, as in many other scleractinian genera, high levels of phenotypic plasticity encrypt species boundaries and complicate the definition of valid taxonomic units (see Todd, 2008). On the basis of skeletal variation, early taxonomists described more than 35 *Pocillopora* species (see Veron & Pichon, 1976), which is likely to be an overestimate. In recent revisions however, several taxa were synonymised due to a lack of clear diagnostic morphological characters reflecting species boundaries, or due to the presence of obvious transitions among morphotypes within an acceptable morphological species range (e.g. Vaughan, 1907, 1918; Hoffmeister, 1925; Wells, 1954; Veron & Pichon, 1976). For example, Veron & Pichon (1976) suggested the extensively studied species *P. damicornis* (Linnaeus, 1758) comprised four intergrading ecomorphs, which were partly defined based on junior synonyms. These comprise: 1) the elongate ecomorph "*bulbosa*" (named after *P. bulbosa* Ehrenberg, 1834) found in very sheltered or turbid deep habitat; 2) an ecomorph from semi-disturbed habitats; 3) the stunted, compact ecomorph "*brevicornis*" (named after *P. brevicornis* Lamarck, 1816) found in exposed habitats and 4) a long and thick branching ecomorph found in temperate regions (Veron & Pichon, 1976). The feature that unifies the aforementioned ecological variants and ultimately defines *P. damicornis* is the absence

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The content of this chapter has been submitted to the Zoological Journal of the Linnean Society of London for publication of true verrucae, i.e. verrucae grade into fully developed sub-branches (Veron & Pichon, 1976).

Molecular studies have provided evidence for the separation of morphological variants within *Pocillopora*, with some confirming a link between morphology and phylogeny (Flot et al., 2008; Schmidt-Roach et al., 2012a, 2013), and others challenging it (Pinzón & LaJeunesse, 2010). From a phylogenetic perspective, Flot et al. (2008) confirmed five distinct genetic lineages in Hawaii that are linked to different morphologies. While most lineages were characterised by individual mitochondrial haplotypes, *P. eydouxi* Milne Edwards & Haime, 1860 and *P. meandrina* Dana, 1846 were resolved only by the nuclear ITS2 region Flot et al. (2008). This observation was mainly attributed to the nature of the mitochondrial coral genome, which evolves slowly compared to other metazoan mitochondria (Shearer et al., 2002; Hellberg, 2006). Its limited inter-specific resolution has often challenged phylogenetic approaches in delineating taxonomical units in coral taxonomy (Shearer & Coffroth, 2008).

At the nuclear level, introgressive hybridisation, incomplete lineage sorting or reticulate evolution further complicates phylogenetic interpretation in some corals (e.g. Oppen et al., 2001; Márquez et al., 2002; Wolstenholme, 2004; Flot et al., 2008; Schmidt-Roach et al., 2012a). Additional lines of evidence are therefore often necessary to resolve taxonomic units. These may include: gross- and fine-scale morphology (e.g. Benzoni et al., 2010; Budd & Stolarski, 2011; Gittenberger et al., 2011; Stefani et al., 2011; Benzoni et al., 2012), symbiont association (Pinzón & LaJeunesse, 2010; Schmidt-Roach et al., 2012a) or reproductive traits (McFadden et al., 2001; Schmidt-Roach et al., 2012a,b).

Using the aforementioned traits in combination, Schmidt-Roach et al. (2012a) showed that the previously reported ecomorphs *sensu lato* Veron & Pichon (1976) within *P. damicornis*, actually represent distinct species rather than morphological variants attributed to local environmental conditions. Mitochondrial molecular phylogenies were found to be congruent with morphological groups within *P. damicornis*, indicating at least five genetically distinct lineages. Nuclear markers on the other hand, recovered only three lineages (Schmidt-Roach et al., 2012a). However, additional information from gross morphology, associated *Symbiodinium* clades, reproductive mode and timing indicated intra-specific diversification patterns that were not revealed by the nuclear DNA data, most likely due to introgressive hybridisation. Most importantly, much of the species' perceived reproductive plasticity could be attributed to cryptic species (Schmidt-Roach et al., 2012b).

Corals often do not meet the criteria of conventional species concepts due to extreme phenotypic plasticity and/or instances of hybridisation along the species genealogical history (e.g. Veron, 1995; Willis et al., 2006). In these cases, the "unified species concept" (USC; De Queiroz (2007)) may represent an appropriate taxonomic approach in delineating valid taxonomic units (Schmidt-Roach et al., 2012a). The USC assumes that species are represented by separately evolving metapopulations whereby criteria associated with previously accepted species concepts are used in synergy to assess metapopulation boundaries. Each criterion represents therefore an independent component in a global line of evidence to support species formation De Queiroz (2007).

In this study we formally revise species within the genus *Pocillopora* by means of morphological analyses and fine-scale structure data from voucher colonies collected from different locations around Australia. In addition to morphological and molecular collected here, we combine molecular, reproductive evidence and symbiont associations inferred from previous studies (Flot et al., 2008, 2010; Pinzón & LaJeunesse, 2010; Schmidt-Roach et al., 2012a,b; Pinzón et al., 2013; Schmidt-Roach et al., 2013) to strengthen species hypothesis. Finally, the identified valid taxonomic units are compared to type material and specimens from taxonomical collections for *Pocillopora* species identification and nomenclature.

Table 5.1: Measurements taken for morphometrical analysis.

	Morphological measurements
1.	The maximal diameter of most distal branchlet/verruca 1mm under tip, at most distal branch
2.	Minimal diameter of most distal branchlet/verruca 1mm under tip, at most distal branch
3.	Distance between most distal branch tip and the base of the most distal ramification of a main branches with secondary branching
4.	Distance between most distal branch tip and the base of the second most distal ramification of a main branches with secondary branching (or base of colony)
5.	Maximal diameter half way between most distal branch tip and the base of the most distal ramification of a main branches with secondary branching
6.	Maximal diameter of branch at this most distal ramification of a main branches with secondary branching
7.	Minimal diameter of branch at this most distal ramification of a main branches with secondary branching
8.	Number of primary branches (branchlets/verrucae) between tip of most distal branch and most distal ramification of a main branches with secondary branching
9.	Length of longest primary branch (branchlets/verrucae) between tip of most distal branch and most distal ramification of a main branches with secondary branching
10.	Number of branches with secondary (or higher) branching originating from investigated branch between tip and 4 cm below tip (or base of colony)

5.2

MATERIAL AND METHODS

5.2.1 Specimen collections:

Seventy-eight colonies/fragments of *Pocillopora* spp. were collected from different habitats throughout its range in Australian waters (Table 1). A fragment of each colony was preserved in ethanol for DNA extraction. Colonies were bleached using 0.5% sodium hypochlorite solution and air-dried for examination of morphology. Type material of the newly described species as well as a selection of specimens was deposited at the Museum of Tropical Queensland, Townsville, Australia.

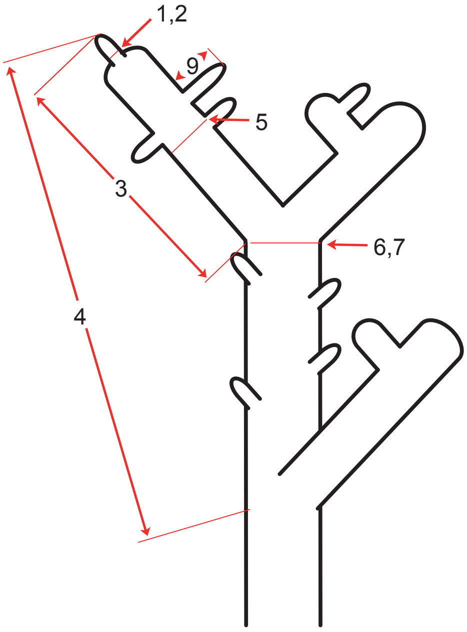

Figure 5.1: Schematic illustration of morphometric measurements taken of corallum. Numbers refer to morphometric measurements taken from each colony (see Table 5.1)

5.2.2 Specimen identification and phylogenetic analysis:

The mitochondrial ORF region (Flot & Tillier, 2007) was sequenced from each colony to ascertain mitochondrial lineage (following protocols in Schmidt-Roach et al. (2012a)). Individuals of a potentially new species identified at Lizard Island were further investigated using the nuclear HSP70B region (following protocols in Schmidt-Roach et al. (2012a)). PCR products were purified and sequenced in both directions for the mitochondrial marker and in reverse direction for the HSP70B marker by Macrogen Inc., Korea. Electropherograms were edited using Sequencher 4.9 (Gene Codes, Ann Abor, Michigan, USA) and aligned manually in BioEdit v7.0.5.3 (Hall, 1999). Sequences generated by Schmidt-Roach et al. (2012a) were used as reference for the sequence alignment (NCBI accession numbers: JX985584–JX985620). MEGA4 (Tamura et al., 2007) was used to generate phylogenetic hypotheses using the Neighbor-joining algorithm under the JC correction (Felsenstein, 1985; Saitou & Nei, 1987) for the mitochondrial ORF region. Network, v4.5.1.6 (http://www.fluxus-technology.com) was used to examine genealogical relationships in the HSP70B region among the newly identified morphotype and those identified by Schmidt-Roach et al. (2012a) using the Median Joining algorithm (Bandelt et al., 1999) (NCBI accession numbers: JX624847-JX624903). The same method was used to compare our data to described genetic lineages of previous studies (including Flot et al., 2008; Pinzón & LaJeunesse, 2010; Souter, 2010; Schmidt-Roach et al., 2012a,b; Pinzón et al., 2013).

5.2.3 Morphometrics:

For morphometric analysis of the identified genetic lineages, a subset of 68 randomly chosen colonies representing all candidate species was analysed, excluding *Pocillopora* Type ϵ due to a lack of skeletal material. Ten skeletal variables (Fig. 5.1, Table 5.1) were measured on each of two branches per colony using an electronic calliper. Branches were selected randomly from the top of the colony. For multivariate analysis a non-parametric permutational MANOVA (i.e. "PERMANOVA") was performed using a two-factor nested model (with genetic lineage as a fixed variable, colony as a random variable, and branch as the lowest level of replicate) in the PERMANOVA add-on package for PRIMER 6 (999 permutations, D1 Euclidean distance resemblance). Discriminant Analysis of Principal Components (DAPC) using *a priori*-defined groups based on the mitochondrial phylogeny was performed to describe clusters of morphologically similar individuals (using the *adegenet*-1.3-5 package (Jombart, 2008). Further, a bi-plot was created using the CDA function in StatistiXL v1.9 (which resulted in a plot identical to the DAPC). Based on the discriminant functions, cluster-based reassignment probabilities were calculated to test for robustness of the identified clusters as well as to estimate levels of separation (Fig. 5.2). In the same matter, individual branch-based reassignment probabilities were calculated to identify intra- and inter-colony variation (Fig. 5.2).

5.2.4 Fine scale morphology:

A subset of 26 colonies was analysed for fine scale morphological differences among genetic lineages using Scanning Electron Microscopy (SEM) (Type: Jeol JSM5410LV). Skeleton samples were cleaned in 70% ethanol and air-dried before vacuum gold coating. The smallest and largest diameter of 6-10 fully developed calices of three colonies per candidate species was measured either by SEM or Light-Microscopy to estimate the size range of calices. Specimens were oriented horizontally for this.

Historical specimens and type material originating from the Ehrenberg collection in Berlin, Lamarck collection in Paris, the international coral collection at the Museum of Tropical Queensland (MTQ) or photos of additional historic samples were examined. Due to a lack of tissue availability for molecular comparisons we could only compare morphological characteristics between our present-day collections and the historical specimens examined. Further, the sampling location of several holotypes examined here (e.g. Ehrenbergs samples) is not recorded, or only the broad geographic regions are reported from which they are collected. This prevented the collection of equivalent fresh samples at the original locations for molecular analysis.

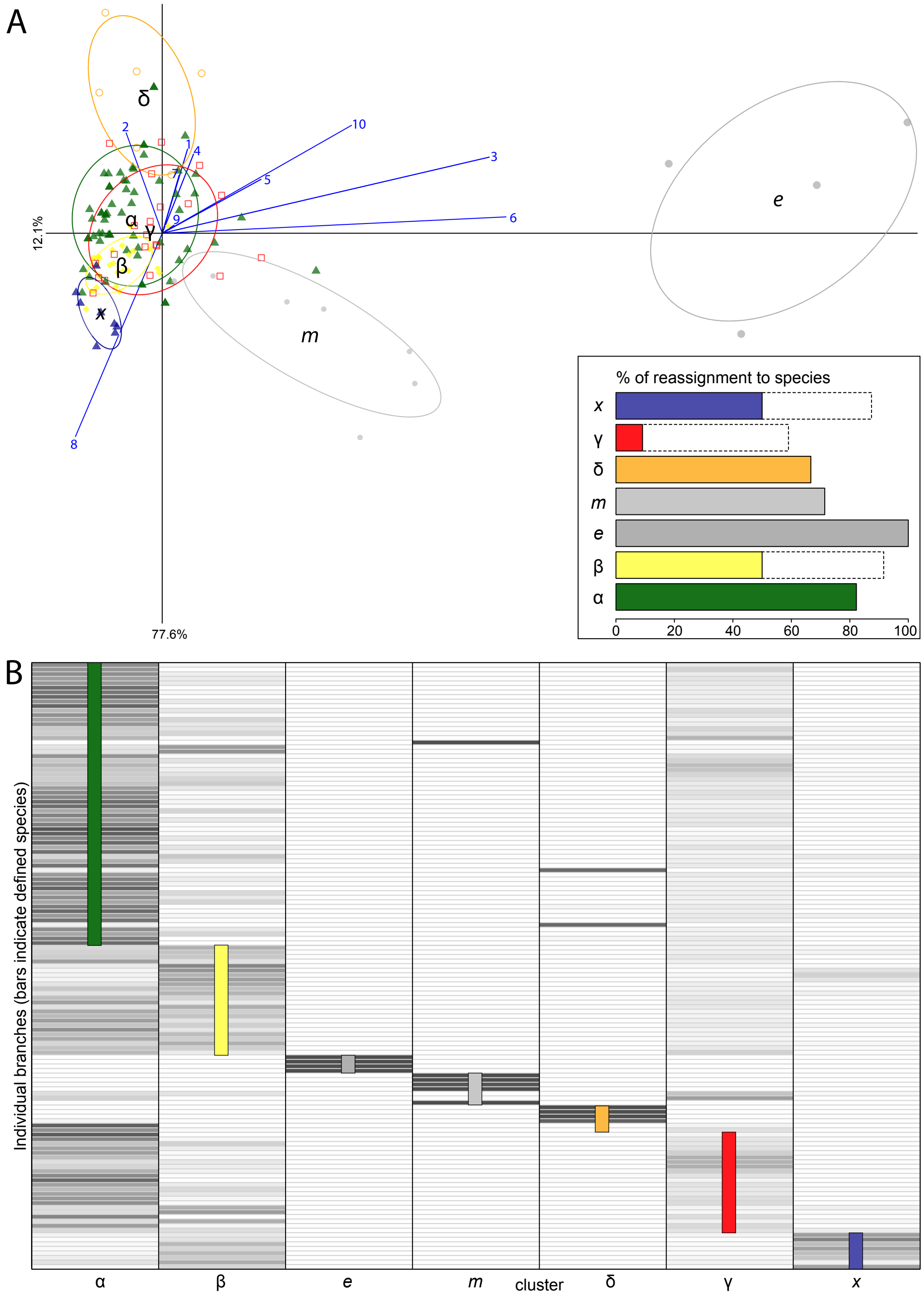

Figure 5.2: A. Discriminant Analysis of Principal Component (DAPC) of gross morphological characters. Bi-plot indicating character contribution shown in blue (see Table 5.1 for explanation of the characters). Individuals represented by dots and groups by elipses. Box: Cluster based reassignment probabilities (dotted lines indicate probabilities if *Pocillopora damicornis* Type α is excluded form the analysis). B. Branch based reassignment probabilities for each cluster, bars indicate genetic lineages (Probability of reassignment of a branch (y-axis) to a certain cluster (x-axis) is indicated by shades: white = 0, dark grey = 1). α. *Pocillopora damicornis*, β. *P. acuta*, δ. *P. aliciae*, γ. *P. verrucosa*, x. *P. bairdi* sp. nov. *e*. *P. eydouxi*, *m*. *P. meandrina*.

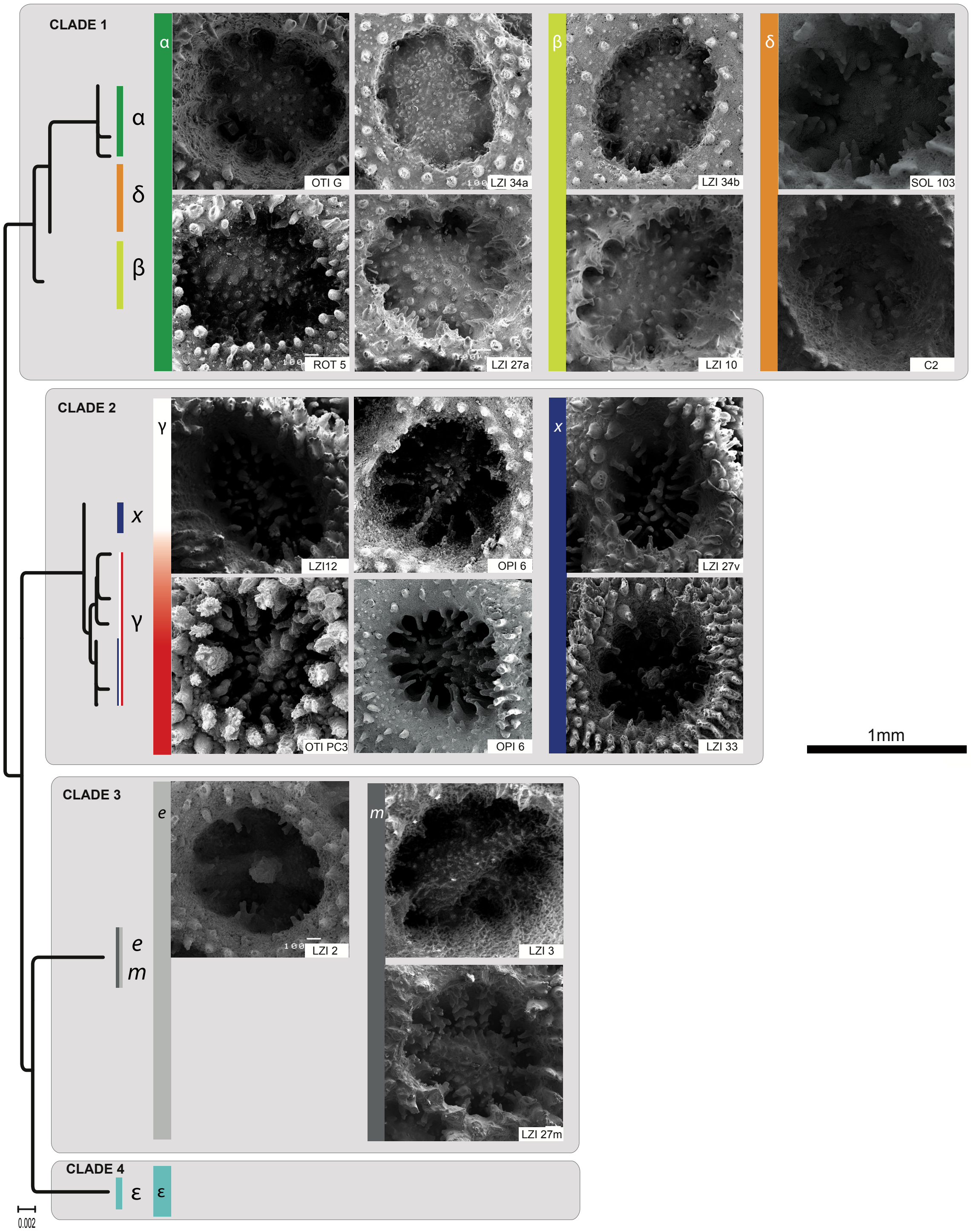

Figure 5.3: Fine scale skeletal differences between genetic lineages. left: phylogeny based on ORF region resolving in four main clades. Right: SEM pictures of different species (bar on the left indicates scale of photos). *α. Pocillopora damicornis*, *β. P. acuta*, *δ. P. aliciae*, *γ. P. verrucosa*, *x. P. bairdi* sp. nov. *e. P. eydouxi*, *m. P. meandrina*, *ϵ. P. brevicornis*

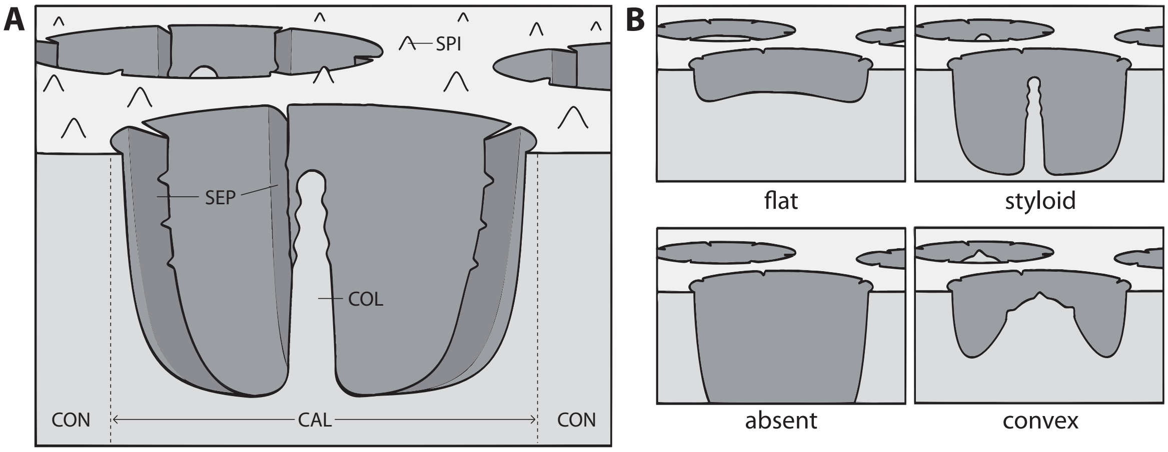

Figure 5.4: Corallite scheme. A. Schematic view on corallite structure (cross-section). CON: coenosteum, SPI: spinulae, CAL: Calice, SEP: speta, COL: columella. B. Schematic illustration of columella differences between the genetic lineages.

5.2.5 Definition of species:

Species are here defined according to the "unified species concept" (USC) proposed by (De Queiroz, 2007), whereby criteria associated with previously accepted species concepts are used in synergy to assess metapopulation boundaries. All available information was considered to assess species integrity for each candidate species including molecular phylogenies, gross- and fine-scale morphology, and where obtainable or known, reproductive and symbiont differences.

5.3

RESULTS

5.3.1 Specimen identification and phylogenetic analysis:

In a previous study, molecular phylogenies inferred from mitochondrial (ORF, COI) and nuclear (HSP70, ITS2) markers together with symbiont association and reproduction were used to identify six different lineages of *Pocillopora*; Type ϵ, Type β, Type α, Type γ and Type δ Schmidt-Roach et al. (2012a). Four of these lineages (Types β, α, δ, ϵ) exhibited distinct morphologies previously attributed to *P. damicornis*, generally defined by the absence of true verrucae (Veron & Pichon, 1976); one lineage (*P. damicornis* Type γ) resembled *P. verrucosa* (Ellis & Solander, 1786) in terms of morphology and another lineage resembled *P. meandrina* morphology. The validity of the genetic lineages identified by Schmidt-Roach et al. (2012a) was recently further supported by Pinzón et al. (2013) sampling various locations throughout the global distribution of the genus *Pocillopora*. Unfortunately, this study was conducted without consideration of morphological characteristics, thus limiting comparisons to species identified here.

The colonies examined in our study encompassed the previously identified genetic lineages within *Pocillopora* in Australia with a final alignment of the mitochondrial ORF region consisting of 67 sequences, 708bp of length (Table 5.2, Fig 5.3), with the exception of one newly identified haplotype that corresponded to a distinct morphotype (x, Fig. 5.3). Lineages were grouped into four main clades (Clades 1–4, Fig. 5.3). Clade 1 consists of *P. damicornis* Type α, *P. damicornis* Type β and *P. damicornis* Type δ described by Schmidt-Roach et al. (2012a). Clade 2 comprises *P. damicornis* Type γ/*P. verrucosa* and a new morphotype (referred to as x). Although individuals of this new morphotype were characterised by a unique haplotype, others exhibited shared haplotypes with *P. verrucosa*/*P. damicornis* Type γ (Fig. 5.3). However, further testing of the nuclear HSP70 region found a >3bp divergence for all sampled individuals of this morph compared to congenerics investigated in Schmidt-Roach et al. (2012a) (including *P. verrucosa*/*P. damicornis* Type γ; Fig. S4.2). Clade 3 comprises *P. meandrina*, a well-distinguished species used by Schmidt-Roach et al. (2012a) for outgroup comparisons against species within *P. damicornis*. Interestingly, the morpho-species *P. eydouxi* shared identical ORF haplotype with *P. meandrina*, confirming previous findings from Hawaii, where only limited support for genetic differentiation in these species based on the nuclear ITS2 region was found (Flot et al., 2008). Clade 4 was previously described by Schmidt-Roach et al. (2012a) as *P. damicornis* Type ϵ.

The aforementioned clades (1 to 4) comprise most of the known genetically distinct lineages within the genus *Pocillopora* as shown in Fig. 5.5, integrating publically available sequences from various geographic locations.

5.3.2 Morphometrics:

Within genetic lineages morphology among types was largely overlapping, except for *P. eydouxi* and *P. meandrina*, which are morphologically distinct from other taxa based on characters 3, 4, 5, 6, 7 and 8 (Table 5.1, Fig. S2.2). *P. eydouxi* has larger branch diameters (>2cm) and greater distances between tip and first ramification (>5cm) and between tip and second ramification (>10cm) than other species. *P. meandrina* is intermediate between *P. eydouxi* and other taxa for these characters (Fig. S2.2). Both *P. eydouxi* and *P. meandrina* have high numbers (>38) of primary branches (branchlets/verrucae) between the tip of the first ramification with secondary branching compared with other taxa. There were some differences among the *P. damicornis* types, in particular *P. damicornis* Type δ had larger distal branchlets/verrucae (2.9–8.3mm) than other taxa. The opposite was true for the newly identified morphotype (x), which had generally smaller distal branchlets/verrucae (0.2–2.6mm) than other taxa.

Nonparametric multivariate analysis identified significant morphological differences among the genetic lineages (PERMANOVA, df=6, *Pseudo*-F= 28.024, p = 0.001, unique permutations = 999), however, there was also significant variation among colonies within a genetic lineage (PERMANOVA, df=61, *Pseudo*-F= 8.6146, p = 0.001, unique permutations = 999)). For the discriminant analysis of the principal components (DAPC) aa-score calculation suggested that all ten principal components of the principal component analysis should be considered to receive maximum resolution of differences between clades. Furthermore, eigenvalues suggested that the first four discriminant functions should be included. DAPC indicated morphological differences between *P. eydouxi*, and *P. meandrina*, *P. damicornis* Type δ and the new morphotype (x), while the remaining groups were overlapping (Fig. 5.2A).

Full reassignment of colonies to their genetic lineages was only observed for *P. eydouxi* (e), while the remaining clusters were moderately to weakly defined (9-82.25%) in the group-based reassignment test (Fig. 5.2B). Reassignment probabilities were lowest for the *P. damicornis* Type γ/ *P. verrucosa* cluster (9%) due to the morphological similarity to *P. damicornis* Type α (Fig 5.2B). The morphometric data indicates high levels of morphological plasticity for *P. damicornis* Type α, including several morphologies similar to other lineages. Thus, the species overlaps in its morphology with several other species and encrypts the clear definition of these groups based solely on gross morphology. Group-based reassignment probabilities of genetic lineages were indeed much higher if *P. damicornis* Type α was excluded from the calculations of the DAPC (*P. damicornis* Type β: 87.5%, *P. damicornis* Type δ: 83.3%, *P. damicornis* Type γ/*P. verrucosa*: 72.7%, *P.* Type *x.*: 62.5%, *P. meandrina*: 85.7%, *P. eydouxi*: 100%; Fig. 5.2 dotted lines). Furthermore, moderate reassignment was observed for *P. bairdi* sp. nov. (x) due to similarity to *P. damicornis* Type β (Fig. 5.2B). In general, reassignment of individual branches to the pre-defined morphological clusters produced similar results to that based on colonies, with the highest reassignment of individual branches to the species *P. eydouxi*, *P. meandrina* and *P. damicornis* Type δ. However, reassignment probabilities did result in some individual branches recovering with higher probability within a different morpho species to that which it was originally assigned, stressing high levels of intra-colony variation and overlap in gross morphological characters among the genetic groups/lineages (Fig. 5.2B).

5.3.3 Fine scale morphology:

Clear fine-scale morphological differences were found among the four genetic clades, and there was little variation among the lineages within each of the clades. Colonies within Clade 1 are characterised by a flat columella ornamented with short spinulae (Fig. 5.4, 5.3). Calices are 0.8–1.4mm in diameter and round to oval. Septa are rudimentary, often only indicated by spinulate septa teeth arranged hexamerally in two equally developed cycles. The coenosteum is ornamented sparsely to densely with short spinulae. Two lineages within Clade 1 (*P. damicornis* Type α and *P. damicornis* β) have identical fine scale morphological features, whereas *P. damicornis* Type δ has a reduced columella in comparison with the other two linages within the clade (Fig. 5.3, 5.8).

Clade 2, comprising *P. verrucosa*/*P. damicornis* Type γ and the new identified morphotype (x) was characterised by absent to styloid columellae, often only indicated by its ornamentation with long spinulae which may be arranged in a line. Calices are round and usually smaller (0.4–0.7mm) than those of Clade 1. The coenosteum is ornamented sparsely to densely with spinluae. Although some variation regarding the development of spinulae covering the strongly reduced columellae as well as the coenosteum can be acknowledged within and between specimens, overall individuals within the clade are very similar in fine structure.

The highest level of intra-clade variation was observed for Clade 3, consisting of *P. eydouxi* (e) and *P. meandrina* (m). The former has a styliform columella, with one to three distinct stylae originating from a diagonally arranged, bridge-like columella. The latter is more variable and characterised by oval-convex to styloid, rarely obsolete columellae, which is consistently more predominant than that of *P. eydouxi.* Both show septa that are hexamerally arranged in two cycles and vary in their development from almost reduced to well-developed, indicated by long spinulae, and where the second septal cycle may be slightly less developed than the first. The coenosteum is ornamented with short spinulae. Calices range 0.5–1.6mm in diameter for *P. meandrina*, and are usually >1mm in *P. eydouxi.*

Unfortunately, material of Clade 4 comprising of *P. damicornis* Type ϵ was limited to DNA and a field photo, thus no skeleton features could be assessed.

5.3.4 Links between genetic/morphological groups and described species

The morphological differences scored among the genetic lineages enabled us to link our taxonomic units with earlier descriptions of *Pocillopora* spp. and formally re-describe species. The observations gathered from previous taxonomic descriptions and the skeleton morphology of type material, showed that the specimens investigated could be classified as belonging to a) four existing species (*P. damicornis*, *P. verrucosa*, *P. eydouxi*, *P. meandrina*), b) one recently described species (*P. aliciae*), c) two species that had previously been synonymised with *P. damicornis* and that

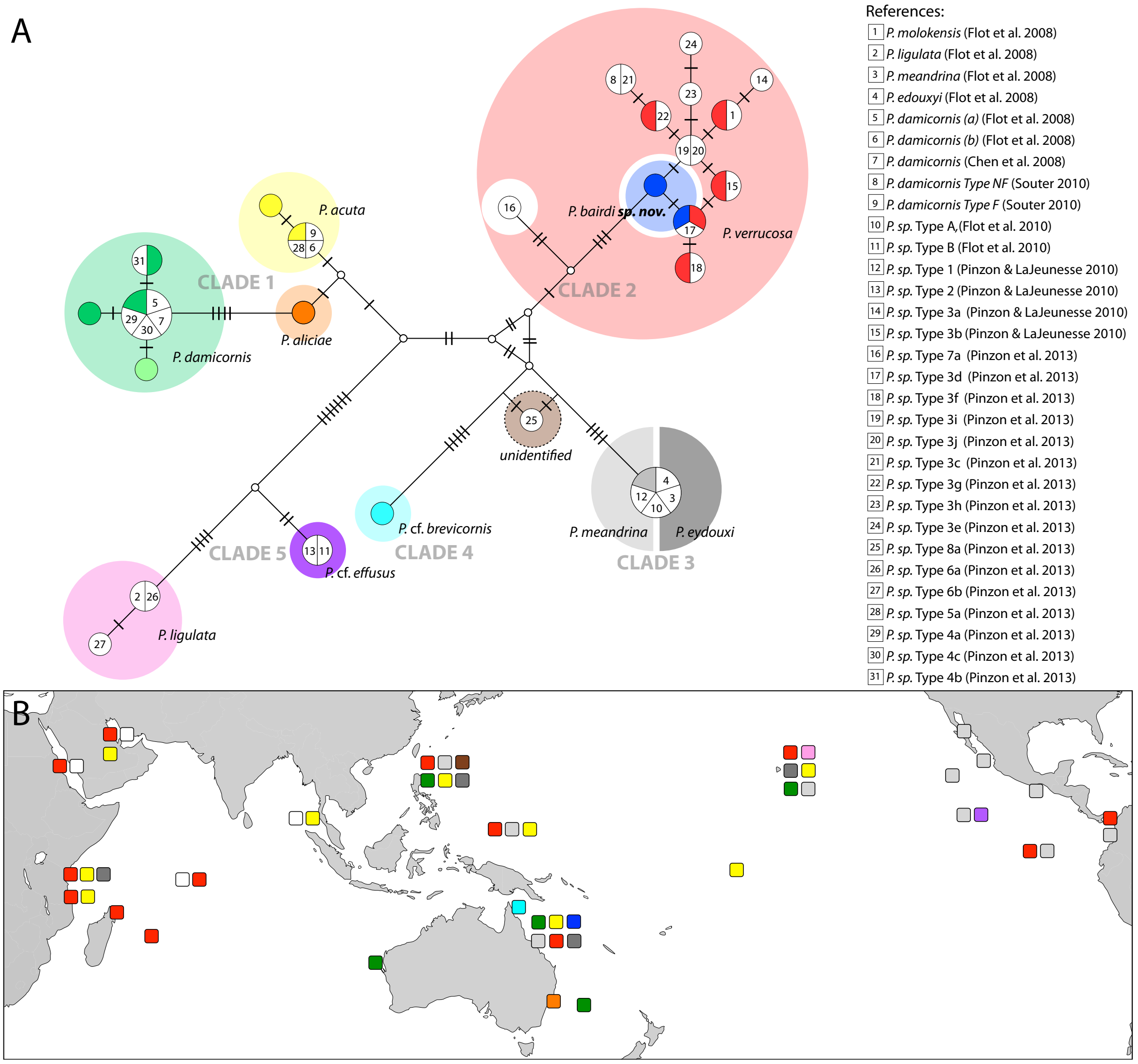

Figure 5.5: A. Haplotype network based on ORF DNA sequence data and incorporating published *Pocillopora* sequence data from other locations across the Indian and Pacific Oceans (total alignment length = 594bp). B. Geographical account of these lineages on a global scale based solely on genetic lineages.

should be resurrected (*P. acuta* and *P.* cf. *brevicornis*), and d) one new species that is here described as *P. bairdi* sp. nov..

Due to the lack of type material for *P. damicornis* (Linnaeus, 1758) (the holotype is temporally lost), corallite structures between our samples and the type specimen could not be compared. However, the original description of *P. damicornis* by Linné (1758) and his illustrations, correspond well with the morphology observed for subtropical *P. damicornis* Type α. Type α shows high levels of phenotypic plasticity (Fig. 5.2) and graduates in its branching from elongate in subtropical to robust, cespitose in tropical regions (Fig. 5.7). Indeed, the apparent differences between latitudinal morphs lead Ehrenberg (1834) to describe the tropical form as a separate species, *P. favosa* (see systematic account). *P. damicornis* Type β matched the holotype of *P. acuta* and agreed well with its columella structure (Fig. 5.9E). The name is derived from its characteristic pointy to sharp branch endings. The species can be divided into two different variations; the elongate *P. acuta* var. *typical* found in sheltered environments and the more compact var. *apiculata* found in exposed environments (derived from Ehrenbergs 1834 species *P. apiculata*, a junior synonym of *P. acuta*). *P. damicornis* Type δ has already been described as *P. aliciae* (Schmidt-Roach et al., 2012a).

Both, *P. damicornis* Type γ and *P. verrucosa* (Ellis & Solander, 1786) share five mitochondrial haplotypes with minor divergence to one another and no clear morphological pattern. Further, nuclear markers failed to recover genetic discontinuities between *P. damicornis* Type γ and *P. verrucosa* (Schmidt-Roach et al., 2012a). Thus we conclude that Type γ and *P. verrucosa* are a single species, morphologically ranging from a typical *P. verrucosa* morphology (i.e. equally sized and distributed verrucae) to a *P. damicornis*-like morphology (i.e. a lack of true verrucae and unequally sized and distributed branchlets; which initially lead Schmidt-Roach et al. (2012a) to differentiate between *P. verrucosa* and *P. damicornis* Type γ). The latter was described as *P. hemprichii* by Ehrenberg 1834 as and is here further referred to as *P. verrucosa* var. *hemprichii*. As the type material of *P. verrucosa* (Ehrenberg, 1834) is also lost to science, a neotype was defined for this species (see systematic account).

Fine scale skeleton structure suggested little differentiation between *P. verrucosa* and the new putative species (x)(Fig. 5.3). However, distinct gross morphology, partial mitochondrial and strict nuclear divergence (HSP70) supported the separation of the new morphotype from *P. verrucosa*. Due to its mismatch with existing *Pocillopora* descriptions, we here describe it as a new species named *P. bairdi* sp. nov..

For clade 3, original descriptions of *P. eydouxi* Edwards & Haime, 1860 and *P. meandrina* Dana, 1846 agree morphologically well with our samples and the fine scale morphological characteristics. Edwards & Haime (1860) illustrated well the styloid columella development of *P. eydouxi*. Thus, the different columella development seems to be of value in differentiating these two species.

The sample of *P. damicornis* Type ϵ corresponded well with the description of *P. brevicornis* Lamarck, 1816. However, due to the lack of skeleton material and additional specimens a formal revival of this species is not possible at this point in time.

5.4 DISCUSSION

We provide clear lines of evidence for morphological differentiation of multiple genetically distinct lineages previously recovered within *Pocillopora damicornis* (Schmidt-Roach et al., 2012a). Mitochondrial molecular phylogenies were congruent with gross morphological groups at the species level. Fine scale skeleton differences supported clade-level and even some species level divergence, confirming the taxonomical utility of fine scale skeleton structure reported for other coral taxa (Benzoni et al., 2010; Gittenberger et al., 2011; Stefani et al., 2011; Benzoni et al., 2012). Comparisons between the morphotypes recovered in this study and type specimens or original descriptions of *Pocillopora* spp. demonstrated that, with one exception (*P. bairdi* sp. nov.), each of these genetically distinct lineages corresponds to previously described species.

Nevertheless, classification of species solely on the basis of skeleton morphology remains unreliable due to high levels of gross morphological plasticity and partial cryptic morphologies. Indeed, colonies or branches within colonies exhibiting an apparent gross morphological transition from one species to another can be found for almost all species within the genus, which leads to taxonomic confusion in the past. In addition, conventional species concepts (i.e biological, phylogenetic) fail to fully resolve species boundaries if applied singularly. For instance, *P. damicornis* and *P. ver-*

rucosa could not be assumed as two biologically or phylogenetically distinct taxa given the limited resolution power of the nuclear regions (ITS2, HSP70), due to apparent occasional introgressive hybridisation (see Schmidt-Roach et al., 2012a). Fine scale morphological analyses on the other hand could strictly resolve both species.

Here we reliably differentiate species within the genus based on a multi-level approach combining morphological and genetic data (incl. data of previous studies on molecular phylogenies Flot et al., 2008; Schmidt-Roach et al., 2012a,b), complimented by findings from previous studies on reproductive traits (Schmidt-Roach et al., 2012a,b) and symbiont association (Pinzón & LaJeunesse, 2010; Schmidt-Roach et al., 2012a) (see below). Thus applying species criteria in synergy as proposed by the "unified species concept" (USC, De Queiroz, 2007) enables reliable differentiation among these species and assessments of the level of speciation.

5.4.1 Species evolution in *Pocillopora*:

The degree of variation found in each of the criteria applied to delineate *Pocillopora* species depicts the genealogical history of the distinct taxa assigned by our approach as well as the mechanisms underlying speciation within the genus. For example, the earliest known fossil records for *P.* cf. *damicornis* suggests a late Eocene origin (>33.9 Ma; (Wells, 1964)). This time frame is sufficient to justify the significant levels of mitochondrial divergence accumulated among lineages of clade 1 (*P. damicornis*, α; *P. aliciae*, δ; *P. acuta*, β). Furthermore, reproduction based on asexual brooding and sexual spawning, constitutes a synapomorphic character for that clade. It is therefore plausible that clade 1 originated from a shift in its reproductive strategy (i.e. ancestral spawning) to a mixed mode of asexual brooding and sexual spawning) leading to reproductive isolation from its con-generic lineages and divergence (Schmidt-Roach et al., 2012b). The same mechanism seems to have induced divergence between the brooding genus *Isopora* and the spawning *Acropora* (Wallace et al., 2007). Seemingly, divergence between *Pocillopora* and the sister genera *Stylophora* and *Seriatopora* is based on the evolution of a different reproductive mode (spawning versus brooding in *Stylophora* and *Seriatopora*; Shlesinger et al., 1998). Further, a miss-match in reproductive timing may have reinforced speciation of *P. acuta* and *P. damicornis* with both maintaining reproductive cycles at opposite lunar phases in the GBR and Hawaii (Richmond & Jokiel, 1984; Schmidt-Roach et al., 2012a). However, introgressive hybridisation suggests that different reproductive traits do not necessarily lead to complete reproductive isolation (e.g. a putative hybrid between *Stylophora pistilata* and *Pocillopora damicornis* was identified at Lord Howe Island; Miller & Ayre, 2004).

In *Pocillopora*, symbiotic algae are transferred maternally and co-evolve with the host, leading to apparent consistent species-specific associations observed over variable geographical scales (Pinzón & LaJeunesse, 2010; Schmidt-Roach et al., 2012a). On the other hand, Bongaerts et al. (2010) identified habitat-specific symbiont associations characterising genetically highly divergent *Seriatopora hystrix*, Dana 1846 populations. Therefore, both host and symbiont, play a role in influencing processes related to the corals niche adaptation, a key process for reproductive isolation and genetic differentiation of a sub-group from the rest of the population. Selective pressure acting at the symbiont level may especially increase isolation of populations in high latitudinal environments by limiting successful migration of less adapted individuals. Indeed, *P. aliciae* at the Solitary Islands and *P. damicornis* at Lord Howe Island are characterised by endemic symbiont associations (Chap. 2, Wicks et al. 2010). However, further research is necessary to understand the impact of the association between specific *Symbio-*

dinium types and host in the speciation process.

Clade 2 comprises *P. verrucosa* (γ) and *P. bairdi* sp. nov. (x), which is characterised by an absent of styloid columella, a character opposed to flat developed columella of species within clade 1. Interestingly, a distinct morphology was not recovered for several mitochondrial haplotypes found within the *P. verrucosa* cluster (Fig. 5.3, excluding the new species described) confirming that high plasticity and fluent phenotypic transitions characterise this species (Chap. 2). Indeed, microsatellite data show that *P. verrucosa* has a homogenous population structure over several thousand kilometres (Pinzón et al., 2013). Fossil records suggest *P. verrucosa* to be of recent origins (Late/Upper Miocene >5.3 Myr) compared to *P. damicornis* (Grigg, 1988). Reticulate evolution is likely responsible for the mitochondrial and nuclear sequence diversity in *P. verrucosa*, i.e. temporally isolated populations may have accumulated sequence divergences, before reticulating into their ancestral lineage by hybridisation. Furthermore, reticulation may explain a distinct mitochondrial lineage described as Type 7 by Pinzón et al. (2013) for the Indian Ocean (Fig. 5.5), which shows no genetic divergence from *P. verrucosa* using microsatellites. The species' gross morphological variation has lead to much confusion in the past; i.e. *P. damicornis* from the Tropical Eastern Pacific is most likely *P. verrucosa* based on molecular phylogenies (Chap. 2). On the other hand, incomplete lineage sorting or introgressive hybridisation seems to support the limited mitochondrial divergence of *Pocillopora bairdi* sp. nov. from *P. verrucosa*, a common phenomenon in the genus (e.g. Combosch et al., 2008; Flot et al., 2008; Schmidt-Roach et al., 2012a, 2013).

Clade 3 comprises *Pocillopora eydouxi* (e) and *P. meandrina* (m). A clear difference in the columella development has been recovered between these two species confirming them both as valid taxonomical units (Fig. 5.3) despite the absence of mitochondrial divergence and the limited differentiation recovered by the nuclear regions (Flot et al., 2008). In addition, Pinzón et al. (2013) found two distinct population clusters within this lineage (Type 1 & 9). Given their fossil age, (Pliocene >2.6 Ma (Felix, 1913; Veron & Kelly, 1988); late Pleistocene > 0.0117 Ma (e.g. López-Pérez, 2012) respectively) and estimated cnidarian mitochondrial mutation rates (0.055% per Ma Hellberg, 2006), we consider the time elapsed since differentiation insufficient for mitochondrial lineage sorting in these two species.

Finally, clade 4 comprises a genetically distinct species likely corresponding to *Pocillopora brevicornis*. However, as mentioned previously, additional samples are necessary to confirm the taxonomic validity of this species.

Species within the genus *Pocillopora* have independent evolutionary histories and directions. This conclusion is supported by microsatellite data finding no indication for recent hybridisation between different *Pocillopora* species (Types 1, 3, 5, 9; Pinzón et al., 2013), a finding that further confirms that these species are stable over time, hence explaining the accumulation of morphological, molecular, and reproductive divergences. However, limited nuclear divergences indicate that rare introgressive hybridisation is likely to occur even between more distant species such as *P. damicornis* and *P. verrucosa*. The exchange of genes responsible for gross morphology during these events may explain the apparent similarity between some of these species. Indeed, interspecific introgression is thought to increase the adaptive potential and reduce the risk of extinction. It may allow for the exchange of beneficial genetic information among species (Seehausen, 2004), e.g. genes supporting adaptation to major environmental shifts. Thus, the apparent genetic transfer at evolutionary time scales between these species within *Pocillopora* may reduce the individual risk of extinction and accelerate evolutionary rates leading to a high potential for adaptation.

In addition to the eight species described here, genetic data indicate that the well-defined morpho-species *P. ligulata* and *P.* cf. *effusus* also represent valid taxa (Flot et al., 2008; Pinzón et al., 2013). Further, the genetically distinct lineage described as Type 8 by Pinzón et al. (2013) from Taiwan (Fig. 5.5) likely represents a valid species. Future research may identify or confirm additional rare species in the genus *Pocillopora*. However, the initially reported species diversity of 17 species (Veron, 2000) may have been overestimated. Further studies using the multidisciplinary approach applied here are needed to test for the validity of such rare species that may have been missed in previous studies.

5.5

SYSTEMATIC ACCOUNT

Family POCILLOPORIDAE Gray, 1840

Genus POCILLOPORA Lamarck, 1816

Diagnosis Hermatypic, plocoid, generally ramose, rarely massive or encrusting; septa are generally poorly developed and mostly arranged in two cycles; the columella is mostly poorly developed (Veron & Pichon, 1976). Verrucae are common, although in some species reduced or absent.

5.5.1 Taxonomical key

Key to studied *Pocillopora* species:

1 Columella weak to well developed, flat and irregular ornamentated with spinulae; mixed mode of brooding and spawning 2

- Columella obsolete, oval convex or styloid 6

2 Corallum cespitose, bushy, irregular sized branchlets 3

- Branches, ascending horizontal from the base, reticulate branching forming flat, plate like appearance, verrucae are obsolete **_Pocillopora aliciae_** 5.5.4

3 Branches with increasing ramification towards the terminal branch end, branch endings blunt, rounded; living phenotype evenly pigmented, often brown or pink 4

- Branches with elongate, pointy to sharp, thin branchlets of various in length; living phenotype pale with characteristic darker pigmentation surrounding oral opening of polyp (brown rings) 5

4 Branches slender, round to flattened . . . . **_Pocillopora damicornis_ (typical)** 5.5.2

- Subterete robust branches with swollen ends **_Pocillopora damicornis_ var. _favosa_** 5.5.2

5 Branches fine, elongate, slender **_Pocillopora acuta_ (typical)** 5.5.3

- Bushy branches (high sub-branching), crowded towards tipps . . . . **_Pocillopora acuta_ var. _apiculta_** 5.5.3

6 Reduced, irregularly developed verrucae; cespitose sub-branching at branch endings. 7

- Verrucae well developed and equally distributed over corallum 8

7 Branches short, thick, even-topped, crowded with irregular but short sub-branching. **_Pocillopora cf. brevicornis_** 5.5.9

- Branches subterete, robust, often swollen ends and commonly growing in high stalks (>40 cm). Verrucae reduced or absent on main stems . . . . **_Pocillopora verrucosa_ var. _hemprichii_** 5.5.5

8 Branches compressed, mostly round and separate; phenotype pale, brown, rarely pink,

darker arround oral opening of polyp; columella obsolete or styloid **9**

- Branches compressed and meandering, robust with very equally sized verrucae; columella styloid or oval convex; often bright pink, blue or yellow, very evenly pigmented . . . **10**

9 Branches spaced, robust (mostly>1.2 cm), mostly cylindrical to terminal branch end. Summits verrucose

***Pocillopora verrucosa* (typical)** 5.5.5

- Branches thin (mostly<1.2 cm), equally thick, flattened towards branch end. Verrucae equally distributed and short (∼1mm), mostly obsolete at summit

***Pocillopora bairdi* sp. nov.** 5.5.6

10 Branches meandering, robust; equally sized, short verrucae covering branches; columella oval convex

Pocillopora meandrina 5.5.8

- Branches spaced, meandering to cylindrical, larger then other species of the genus, very reduced sub-branching; verrucae, evenly sized, and obsolete on summits; columella styloid

Pocillopora eydouxi 5.5.7

5.5.2 *Pocillopora damicornis* (Linnaeus, 1758)

(Figs 5.6, 5.7)

Synonymy

Millepora damicornis β Linnaeus, 1758 p. 791

Madrepora damicornis Pallas, 1766 p. 334, Esper (1791) pl. 46 & pl. 48

Pocillopora favosa Ehrenberg, 1834 p. 351

Taxonomic History Linné (1758) based his description of *Pocillopora damicornis* on three references: Gualtieri (1742) pl. 31 (see Fig. 5.6 H), Rumphius (1741) p. 243 (which shows a specimen of *Seriatopora hystrix*, later described by Dana (1846)), and Bauhin (1650) p. 846 (suggested to show *Millepora alcicornis* by Boschma (1948)). Initially described as *Millepora damicornis* by Linné (1758), the species was placed in the genus *Madrepora* by Pallas (1766), and subsequently into *Pocillopora* by Lamarck (1816). The genus *Pocillopora* was erected by Lamarck based on *Pocillopora acuta*, a taxon later synonymised under *P. damicornis* (Veron & Pichon, 1976). Three other species (*P. bulbosa, P. brevicornis, P. cespitosa*) were additionally synonymised under *P. damicornis* (Veron & Pichon, 1976), all considered to be morphological variants of a single taxon associated with different environments. Unfortunately the type specimen of *P. damicornis* is temporarily lost to science. The specimen is part of the royal invertebrate collection of Lovisa Ulrika of Prussia, Queen of Sweden which is now curated by the Uppsala University Museum of Evolution, Uppsala. Linné examined her collection ("Museum Ludovicae Ulricae"), housed at the castle of Drottingholm near Stockholm, on several occasions to describe these specimens, later partly published in the 10th edition of Systema Naturae (Linné, 1758) (Wallin, 2001). However, Linné produced no labels or inscriptions for the specimens making subsequent identification of the type specimens extremely difficult (Wallin, 2001). Linné adopted the name "*damicornis*" from Gualtieri (1742) who labeled his illustration: "damae cornua". Considering Linné's description of branches "formed like Fallow Deer antlers" (a deer species he described himself; *Dama dama* Linneaus, 1758), it is likely that he examined a specimen of *P. damicornis* Type α Schmidt-Roach et al. (2012a) which has antlers shaped branching in some geographical locations (Fig. 5.7 q-u). However, the species has been the subject of much confusion, particularly the differentiation between *P. bulbosa* and *P. damicornis*. Hoffmeister (1925) synonymised *P. bulbosa* Ehrenberg, 1834 and *P. ceaspitosa*

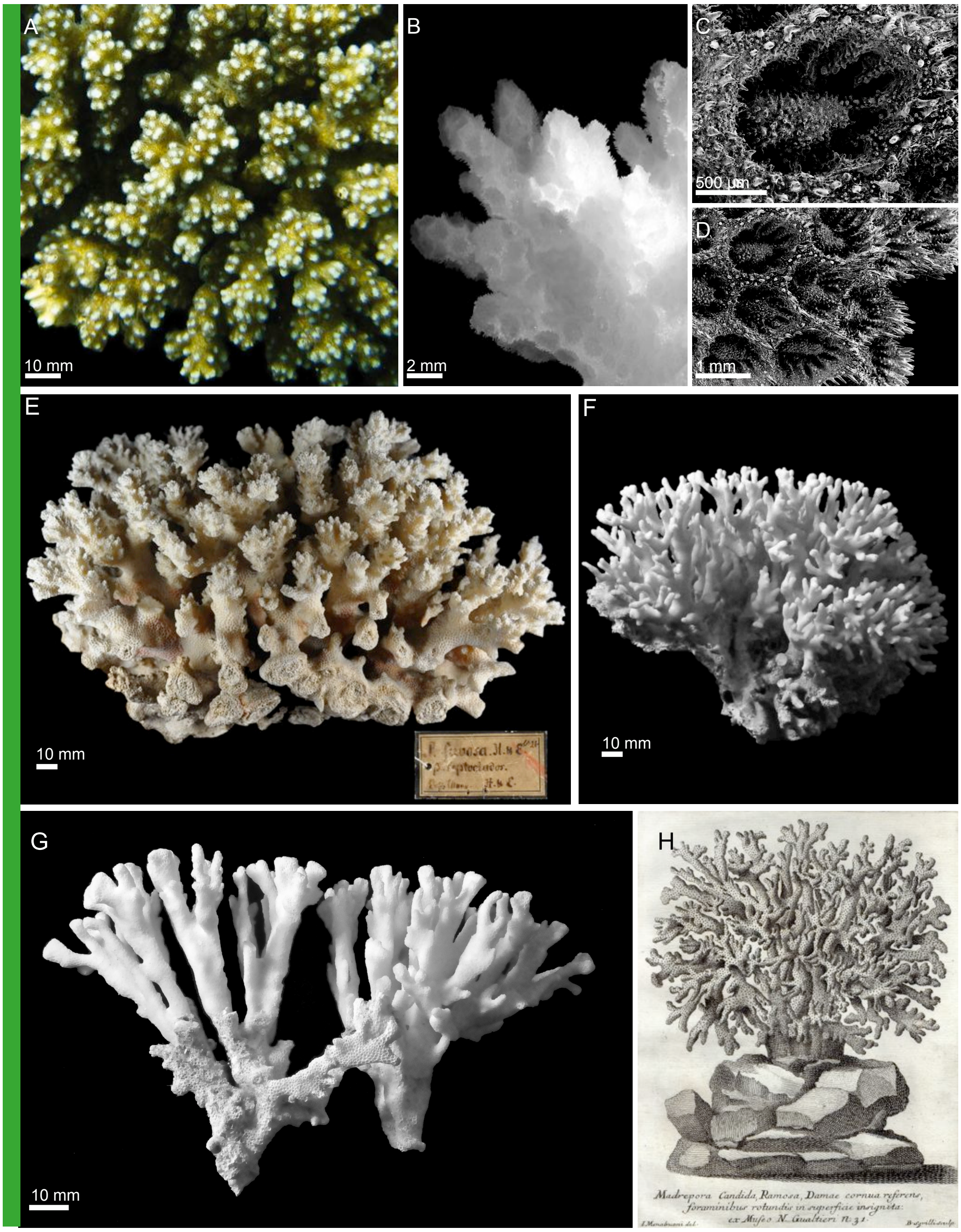

Figure 5.6: *Pocillopora damicornis* **A.** *In situ.* **B.** Skeleton. **C–D.** SEM photos (Photos: Paul Muir. **E.** Corallum of type specimen of *P. favosa* Ehrenberg, 1834 (side view). **F–G**. Elongate morphs of *P. damicornis* (side view). **H**. First illustration of *P. damicornis* by Gualtieri (1742).

Dana, 1846 as varieties of *P. damicornis*. The most recent revision of these taxa supported this idea but differentiated four ecomorphs, including *P. brevicornis* and *P. bulbosa* as varieties of *P. damicornis*, all considered habitat specific morphological varieties of a single phenotypically plastic species (Veron & Pichon, 1976).

Holotype. Temporarily lost to science. Last reported in the castle of Drottingholm near Stockholm (Wallin, 2001).

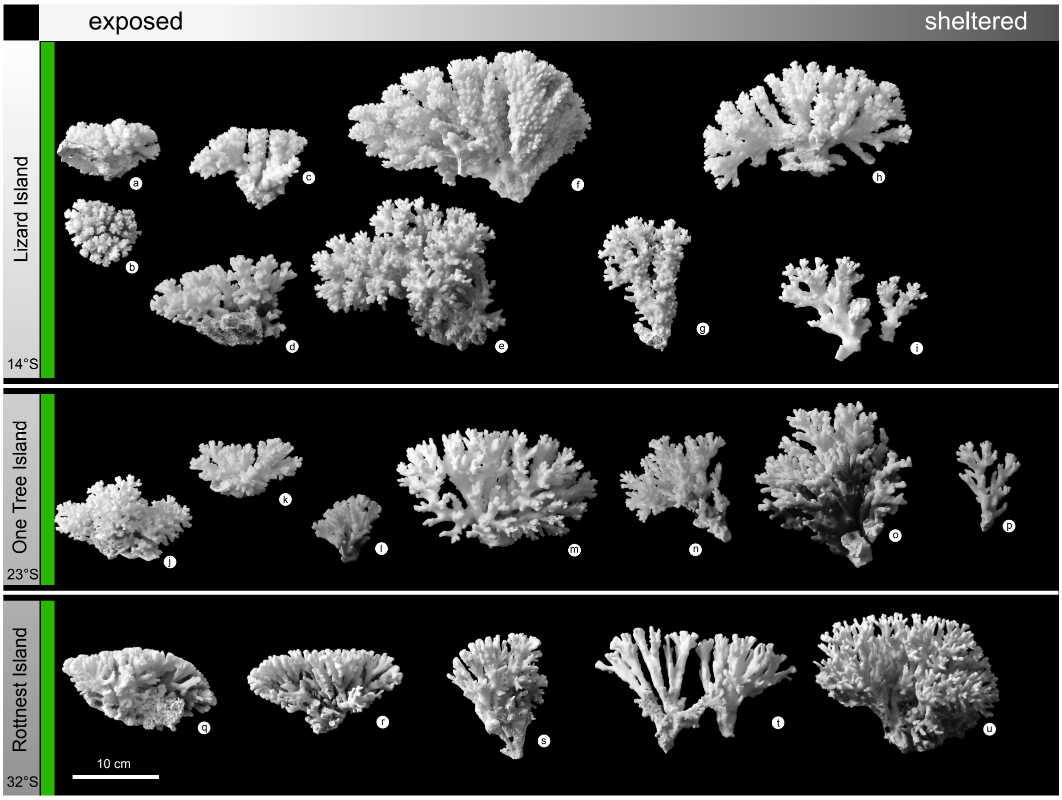

Figure 5.7: Illustration of gross morphology plasticity of the corallum of *Pocillopora damicornis* in different environments and at different latitudes (side views).

Material studied. *MTQ samples:* G33397 (AIMS MCC) Wheeler Reef, Australia (18°48'S 147°32'E); G33366 Bowl Reef, Australia (18°31'S 147°32'E); G33365 North-West Reef, N of Thursday Island, Australia (10°33'S 142°15'E); G33622, G33628 Shrimp Reef, QLD, Australia (18°53'N 145°05'E). G33618 Bewick Island, QLD 0-12 m (14°26'S 144°49'E); G3397 Wheeler Reef, QLD, (18°48'S 147°32'E). Further material: Lizard Island (11 specimens), Rottnest Island (6 specimens), One Tree Island (10 specimens).

Corallum: Compact to elongate, very plastic in its branching, and rounded branch endings. Two morphological variants can be differentiated, which are found at different latitudes. *Pocillopora damicornis* var. *typical*. Mostly subtropical, elongate, slender, partly flattened branching, verrucae obsolete (Fig. 5.7 p-u). *Pocillopora damicornis* var. *favosa* is found in lower latitudes in Australia (> 20°S). The corallum is cespitose, rather stout, with increased ramification towards the apex (Fig. 5.7 a-i). Main stems are robust and range in height from short in exposed environments to a more cespitose, elongate growth with higher ramification of the main stems in sheltered environments. There is a gradual gradation between these morphological variants in Eastern Australia from the tropics to the subtropics (Fig. 5.7 j-p).

Corallites and coenosteum: Calices are between 0.8–1.4mm in diameter mostly round, but can be oval towards the branch endings. The columella is flat and ornamented with short spinulae, septa are rudimentarily often only indicated by spinulate septa teeth. The coenosteum is ornamented sparsely to densely with short spinulae.

Colour and pigmentation of the live colony: Phenotype evenly pigmented (often pink to brown, rarely green).

Habitat and Biology. In the Central and Northern Great Barrier Reef this species is common on the exposed side of reefs in high-energy environments. It was observed from lagoons, back reef habitats and the deeper habitats of the reef slope (> 8m), but is most abundant at the reef crest were it occurs partly in sympatry with *P. acuta* (see mosaic colony Fig. 5.8). In the Southern GBR this species is very abundant and occurs in most habitats. *P. damicornis* has a mixed mode of reproduction. It reproduces asexually through the production of brooded larvae (Stoddart, 1983; Ayre & Miller, 2004; Sherman et al., 2006) and reproduces sexually by broadcast spawning gametes (Schmidt-Roach et al., 2012b). It releases brooded larvae around full moon on the Great Barrier Reef (Tanner 1996; pers. observation), but releases brooded planulae close to new moon in Western Australia (Ward, 1992).

Distribution. Pacific Ocean, Indian Ocean. Specimens were identified from various locations along the Great Barrier Reef down to the subtropical reefs of Lord Howe Island, as well as at subtropical locations in Rottnest Island in Western Australia. Sequences from public databases indicate a Indo-Pacific distribution of this species from Taiwan, Hawaii to the Indian Ocean; it seems to be absent in the Tropical Eastern Pacific and possibly the Western Indian Ocean as the genetic lineage could not be identified in the region (see Flot et al., 2010; Pinzón & LaJeunesse, 2010; Pinzón et al., 2013) (Fig. 5.5).

5.5.3 *Pocillopora acuta* Lamarck, 1816

(Fig 5.9, 5.10)

Synonymy

Pocillopora acuta Lamarck, 1816 p. 274

Pocillopora bulbosa Ehrenberg, 1834 p. 351 (p 127)

Madrepora damicornis γ Pallas, 1766 p. 334, Esper (1791) pl. 46A

Pocillopora apiculata Ehrenberg, 1834 p. 351

Pocillopora cespitosa Dana, 1846 p. 525, pl. 49, fig. 5, 5a

Pocillopora subacuta Edwards & Haime, 1860 p. 303

Taxonomic history. *Pocillopora acuta* Lamarck (1816) defines the genus *Pocillopora* (Type specimen Fig. 5.9 E). Esper (1791) illustrated a colony of Pallas's (1766) *Madrepora damicornis* type γ (one of three morphological variants identified by Pallas; Fig. 5.9 F) as an elongate, fine branching morph, later in literature commonly referred to as Ehrenberg's 1834 species *Pocillopora bulbosa.* Dana's (1846) interpretation of this elongate morphology as *P. bulbosa* lead to much confusion in consecutive descriptions. However, Lamarck (1816) referred to Esper's (1791) illustration as representing *P. acuta.* The type specimen of *P. bulbosa* (a relative small fragment) shows a compact morphology and does not match what has been referred to as *P. bulbosa* in literature. Further, Ehrenberg's collection contains a specimen labeled *P. acuta*, which matches Lamarck's description. Thus, *P. bulbosa* represents a junior synonym of *P. acuta.* In addition, Ehrenberg (1834) described a moderately compact morph of this taxon as *Pocillopora apiculata* (Fig. 5.9 H). Dana (1846) described and illustrated this species as *P. cespitosa* (Fig. 5.8 D).

Holotype. MNHN-IK-2010-792 (Fig. 5.9 E). Origin: Indian Ocean.

Material studied. *MTQ samples:* G37619 China Sea, Pratas Reef (21°50'N 117°00'E); G33370 Orpheus Island, (18°36'S 146°29'E); 51948 Gulf of Aden, Yemen (12°47'N 045°03'E); G33627 Shrimp Reef, QLD Australia (18°53'S 148°05'E); G33376 Fantome Island (Palm Islands), QLD Australia (15-20 m) (18°41'S 146°31'E). G33375 Houghton Island, QLD, Australia (14°31'S 144°58'E). G35114 Flinders Reef (Coral Sea), 5 m (17°40'S 148°20'E). Further

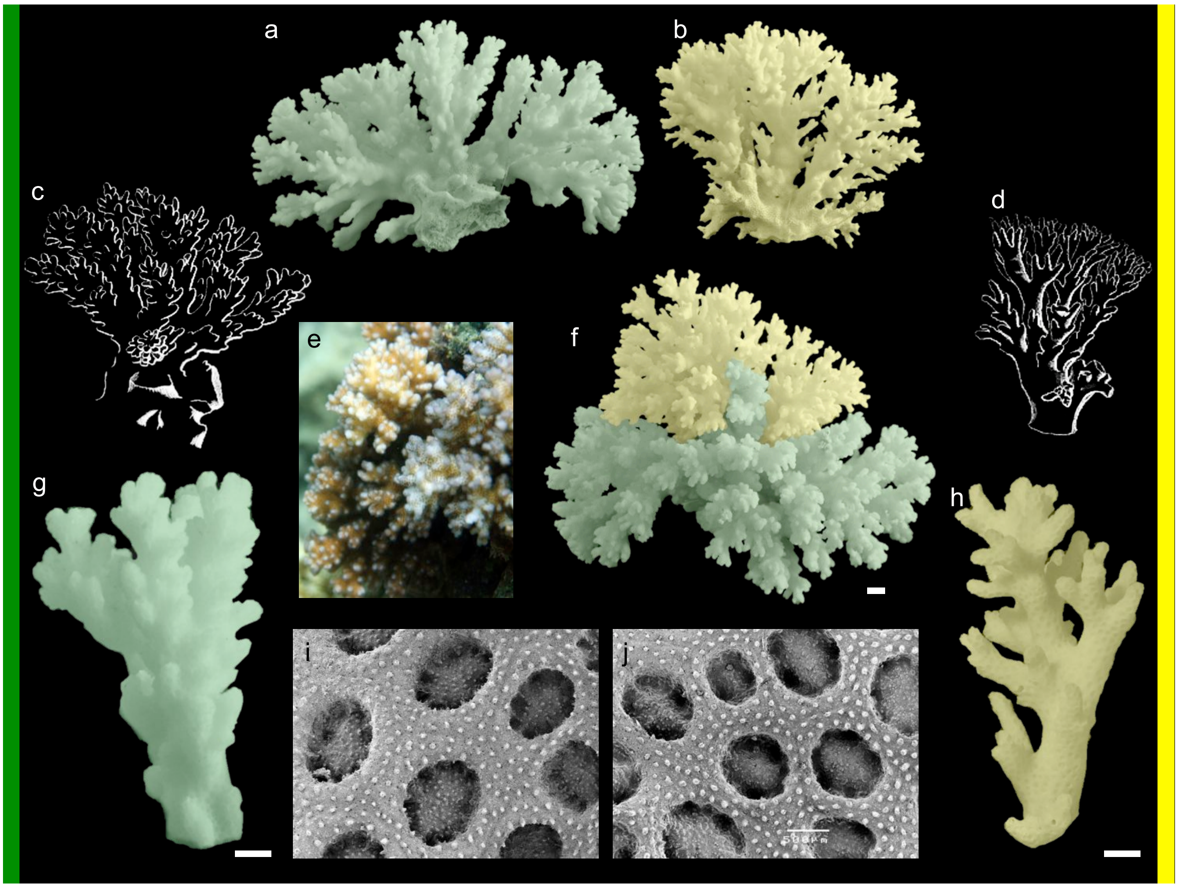

Figure 5.8: Mosaic colony of *Pocillopora damicornis* (green) and *P. acuta* (yellow). **c, d** Inverted illustrations modified from Dana (1846) of *P. damicornis* (c) and *P. ceaspitosa* (d). **e** Field appearance of these species next to each other. **f** Corallum of colonies of *P. damicornis* and *P. acuta*. **g, h** Branches of colonies. **i, j** SEM of colonies.

material: Orpheus Island (2 specimens), Lizard Island (12 specimens).

Corallum: Compact in exposed environments to elongate in sheltered environments ((Fig. 5.10). Cespitose, much branched, branches mostly round and rarely flattened. Consistently pointy branches, sharp tips. Two morphological variants can be differentiated, which are found in exposed and sheltered environments respectively. *P. acuta* (typical) is characterised by elongate, fragile, slender branches almost approaching *Seriatopora hystrix* (Fig 5.10 e-j) but no seriate cells; its found in sheltered environments. *P. acuta* var. apiculata resembles *P. apiculata* Ehrenberg, 1834. Corallum compact to compressed, but still cespitose with crowded branches (Fig. 5.10 a-d).

Corallites and coenosteum: Calices are between 0.7–1.3mm in diameter, often oval due to the narrow, slender growth. The columella is flat and ornamented with short spinulae, septa are only rudimentarily developed, often only indicated by spinulate septa teeth and arranged hexamerally in two equally developed cycles. The coenosteum is ornamented sparsely to dense (mostly towards the branch endings) with short spinulae.

Colour and pigmentation of the live colony: Pale (sometimes greenish) with characteristic darker pigmentation surrounding oral opening of polyps (giving appearance of brown rings outlining polyps).

Habitat and Biology. In the Central and Northern Great Barrier Reef this species is common on the leeward site of the reefs, but compact morphs can also be found in the exposed environments. It occurs in lagoons, back reef habitats to the deeper (> 12 m) habitats of the reef slope. In sheltered habitats the fine morphology is exhibited. At One Tree Island in the southern GBR this species was not recorded. *P. acuta* releases brooded asexual larvae after new moon (Schmidt-Roach et al., 2012a) supporting earlier observations of brooding in this species (Marshall & Stephenson, 1933). Although it has not been observed to spawn, it is expected to have a mixed mode of reproduction as observed in its sister species *P. damicornis* (Schmidt-Roach et al., 2012b).

Distribution. Distribution: Specimens were identified from various locations along the Great Barrier Reef, however, no colonies were observed at One Tree Island in the Southern GBR. Sequences from public databases indicate a wide distribution of this species reaching from the central Pacific to the Indian Ocean; it seems to be absent in the Tropical Eastern Pacific as the genetic lineage was not identified in the region (see Flot et al., 2010; Pinzón & LaJeunesse, 2010; Pinzón et al., 2013) (Fig. 5.5).

5.5.4 *Pocillopora aliciae* Schmidt-Roach et al., 2013

(Fig. 5.11, 5.12)

Taxonomic history. *Pocillopora aliciae* was previously considered a temperate ecomorph of *P. damicornis* (e.g. Veron & Pichon 1976), but was described as a separate species in Schmidt-Roach et al. (2013).

Holotype. MTQ-G65423. Black Rock, off South Solitary Island, NSW, Australia (30°12'0.55"S 153°15'27.05"E).

Other material studied. *MTQ samples:* G65424 (Paratype) same as holotype. G65425 Smoky Cape, NSW, Australia (30°54'22"S 153°5'9"E). G65900 Bryon Bay, NSW, Australia.

Corallum. *Pocillopora aliciae* is characterised by its robust, almost horizontally branching. Small sub-branches arise vertically from the main branches, but are generally short, giving the colony an overall flat appearance. Colonies seldom exceed 30 cm in diameter. Branch endings are rounded and verrucae reduced to entirely absent.

Corallites and coenosteum. Calices are 0.8–1.1mm in diameter, the columella is weakly developed and flat, ornamented with short spinulae. The septa are hexamerally arranged in two cycles and weakly developed, often only indicated by spinulae. The coenosteum is ornamented with short spinulae.

Colour and pigmentation of the live colony. Green.

Habitat and Biology. The species was observed on rocky habitats in depths between 2-32 m. *P. aliciae* releases brooded larvae after full moon (Schmidt-Roach et al., 2012a). Although it has not been observed to spawn, it is expected to have a mixed mode of reproduction as observed in its sister taxon *P. damicornis* (Schmidt-Roach et al., 2012b).

Remarks. See Schmidt-Roach et al. (2013) for a more detailed description.

Distribution. The species has only been recorded on the East Coast of New South Wales, Australia, from Byron Bay to Port Stephens. Genetic samples analysed originated from the Solitary Islands.

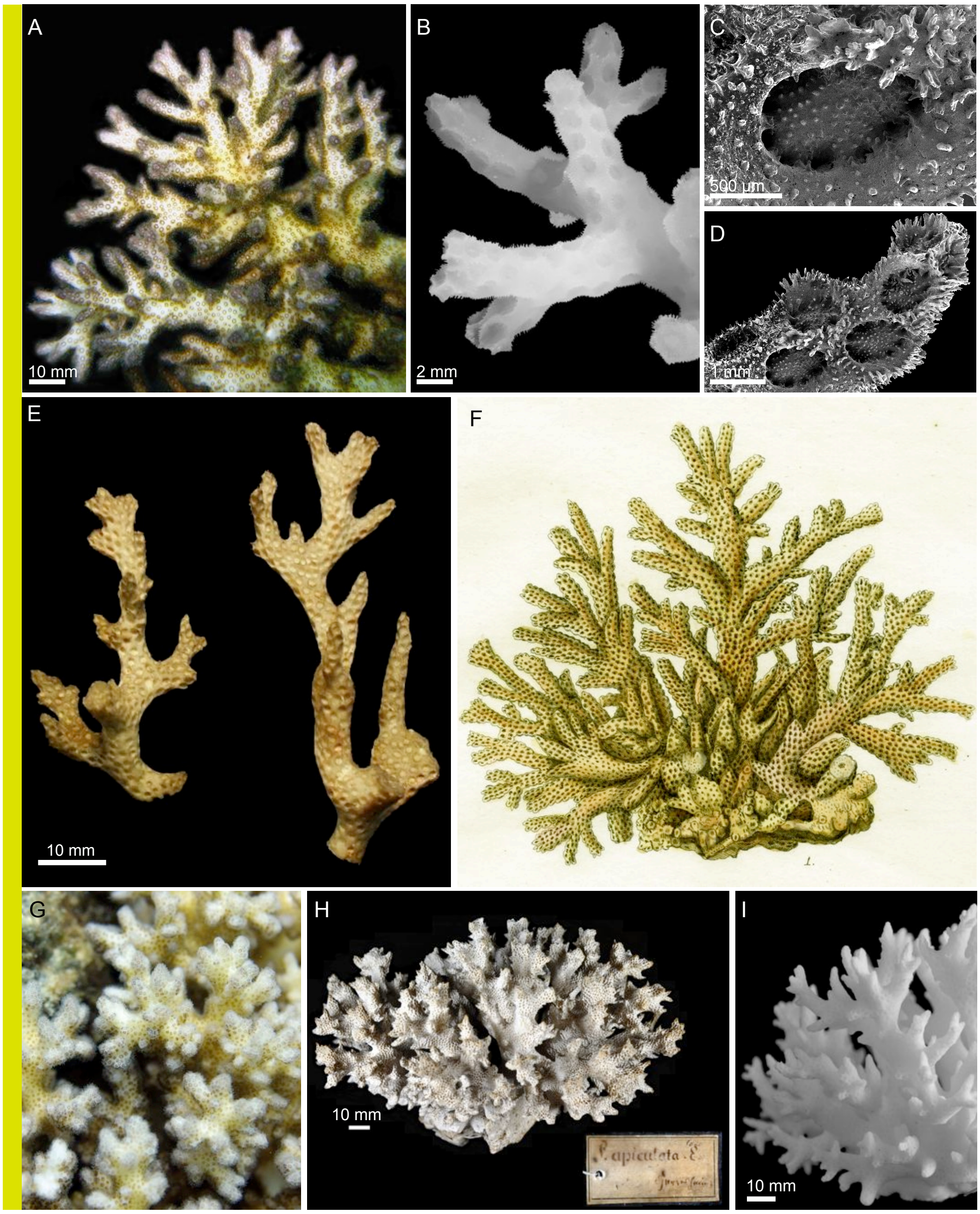

Figure 5.9: *Pocillopora acuta*. **A.** *In situ* appearance. **B.** Skeleton of specimen. **C-D.** SEM of specimen (Photos: Paul Muir). **E.** Side view of corallum of holotyp of *Pocillopora acuta* Lamarck, 1816 (Photo: Michel Pichon). **F.** Drawing by Esper (1791). **G.** *P. acuta* variation apiculata morph *in situ*. **H.** Holotype of *Pocillopora apiculata* Ehrenberg, 1834. **I.** Skeleton of compact morphology of *P. acuta*.

5.5.5 *Pocillopora verrucosa* (Ellis & Solander, 1786)

(Fig. 5.13, 5.14, 5.15)

Synonymy

Madrepora verrucosa Ellis & Solander, 1786 p. 172

Pocillopora hemprichii, Ehrenberg, 1834 p. 352

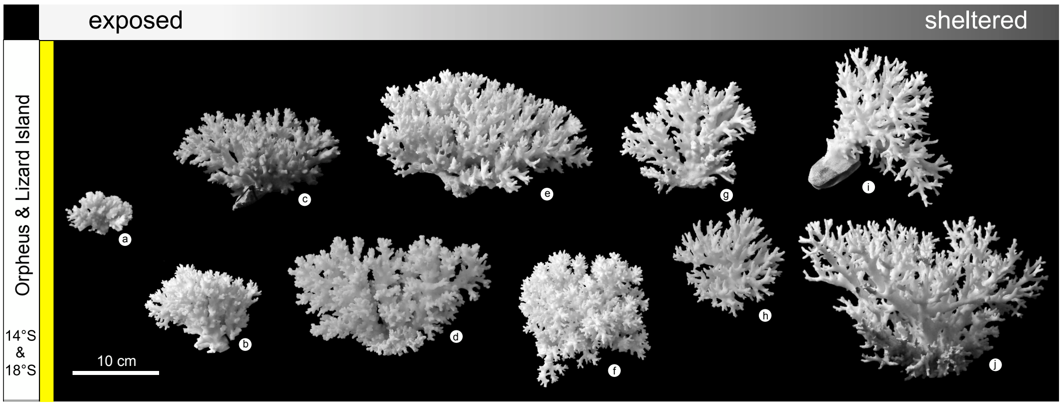

Figure 5.10: Illustration of gross morphology plasticity of the corallum of *Pocillopora acuta* in different environments (side views).

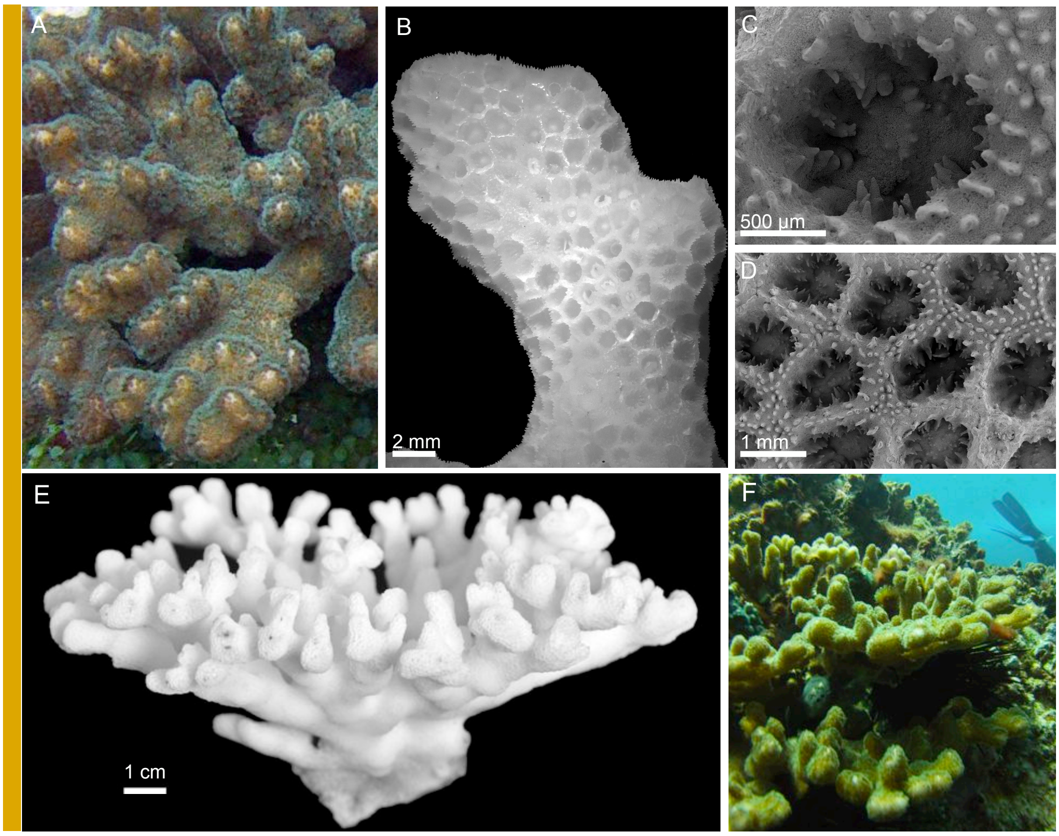

Figure 5.11: *Pocillopora aliciae*. **A.** Field appearance. **B.** Skeleton. **C-D.** SEM of corallite structure. **E.** Corallum of holotype (Schmidt-Roach et al., 2013). **F.** Typical growth from on reef slope.

Pocillopora danae Verrill, 1864 p. 59
?Pocillopora molokensis Vaughan, 1907 p. 91

Taxonomic history. *Pocillopora verrucosa* was described by Ellis & Solander (1786). Together with Joseph Banks, Solander joined the Endeavour voyage under Captain Cook. Returning from the Providential Channel in Northern Queensland, Australia, Banks (1770) notes in his journal the collection of "many curious fish and mollusks

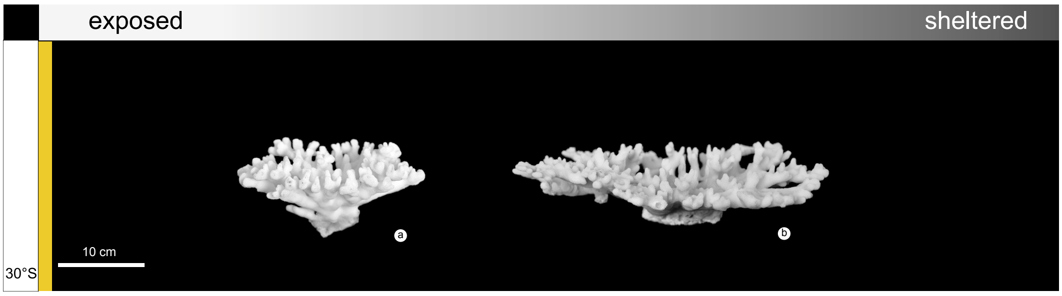

Figure 5.12: Illustration of gross morphology plasticity of the corallum of *Pocillopora aliciae* (side views).

besides corals of many species" (see Beaglehole & Banks, 1962). After return to England, specimens collected at this and other locations visited by the HMS Endeavour were examined and described by Ellis & Solander (1786). Unfortunately, the type specimen could not be located in the Hunterian collection of the Museum of the University of Glasgow, which curates the corals described by Ellis and Solander. Eighteenth century specimens are largely unlabelled and confirmation of samples originating from Ellis and Solander is based on their illustrations, which are not known to exist for *P. verrucosa*. Due to this loss, a neotype is here defined from a geographic region likely to be the origin of the type specimen. The neotype was sampled at Lizard Island, which was visited by the Endeavour on the 12th of August 1770.

Holotype. Hunterian Museum in Glasgow was contacted, but a type could not be identified and is thus considered lost.

Neotype. MTQ-G65923 Lagoon, Lizard Island, QLD, Australia (14°41'14.94"S 145°27'57.42"E). 2 m, (19.11.2011) (Coll. S. Schmidt-Roach) (Fig. 5.12 g-i)

Other material studied. *Pocillopora verrucosa* (typical): MTQ-samples: G33427 Tijou Reef, QLD, Australia (13°10'S 143°57'E) 0-10 m; G33405 same as before (1-2 m); G33424 Great Detached Reef, QLD, Australia, 0-15 (11°42'S 144°00'E); G33422 Bowl Reef QLD, Australia (0-10 m) (18°31'S; 147°32'E). G33417, G33619 Hope Island, QLD Australia (0-3 m); G46830 South China Sea, Pratas Reef (20°35'N 116°46'E); G43835 Philippines, Coron Island, Calis Point (11°34'N 120°07'E), *Pocillopora verrucosa* var. *hemprichii*: USNM No. 696 Type specimen of *Pocillopora danae* Verril, 1864 (based on photograph by Vaughan (1918)). MTQ samples: G33384 Great Detached Reef, QLD Australia 0-1 m (QLD 11°48'S 144°03'E); G33383 AIMS site no. 33; G35117 Flinders Reef (Coral Sea), QLD, Australia (17°40'S 148°20'E). G33392 AIMS site no. 33. G33393 QLD. Further material: Orpheus Island (4 specimens), Lizard Island (11 specimens), One Tree Island (1 specimen).

Skeletal characteristics of the neotype. The corallum measures 188mm in length, 161mm in width and 145mm in height. The corallum is hemispherically cespitose, with spaced (~8–10mm between branches), robust and almost straight branching (diameter of main branches mostly >12mm). Two-dimensional ramification of two or more branches at branch tips may give some branches a flat appearance. Verrucae are equally distributed but irregular in size and shape, summits are verrucose. Calices are mostly round and usually smaller (approx. 0.4–0.7mm) than those of *P. damicornis*, *P. acuta* and *P. aliciae*. The columella is absent to styloid, mostly just indicated by its ornamentation with long spinulae, which may be arranged in a line; septa are only rudimentarily developed, often only indicated by irregular, but long (~100–150μm) spinulate septa

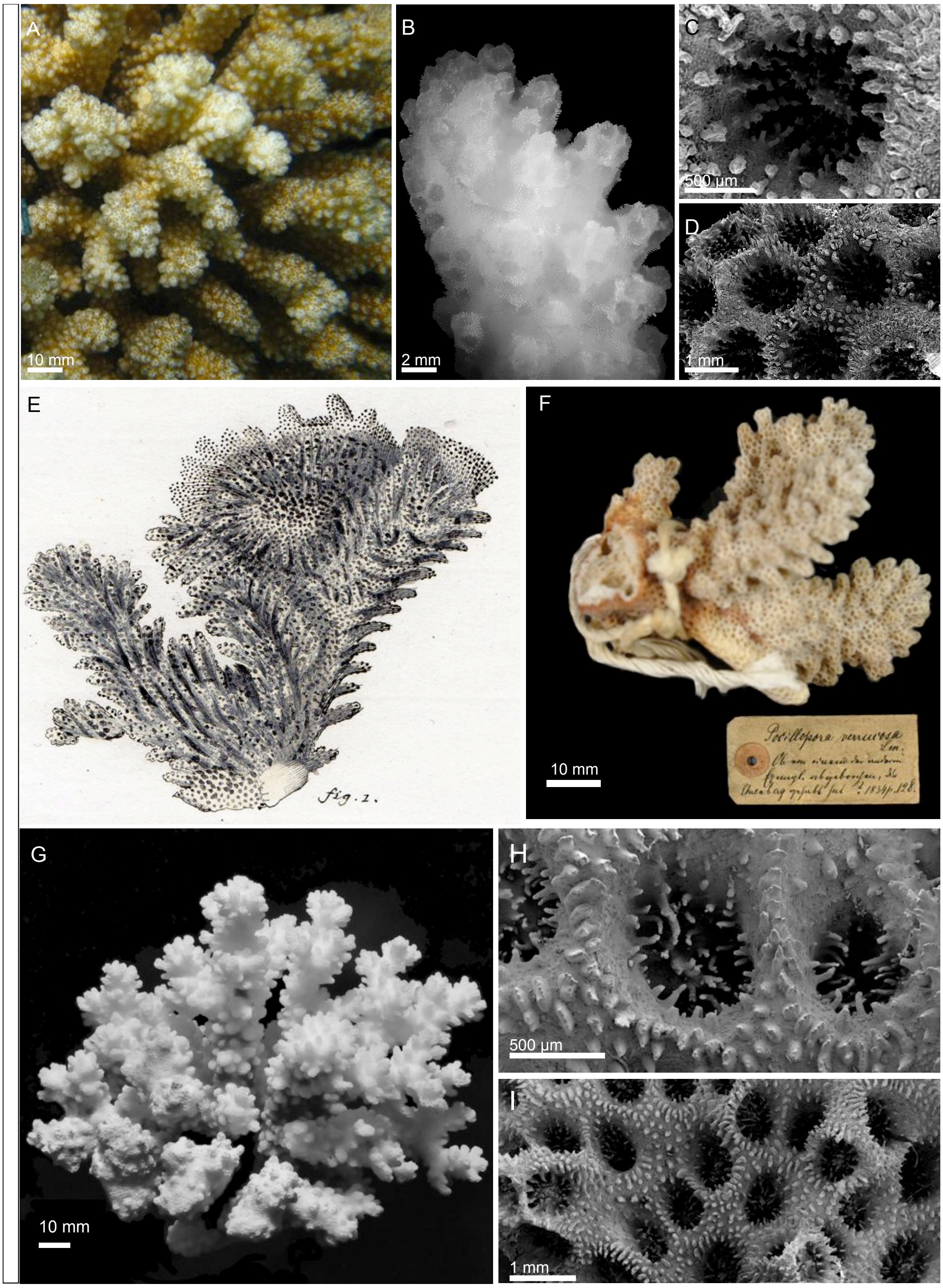

Figure 5.13: *Pocillopora verrucosa* (typical). **A.** *In situ.* **B.** Skeleton of branch. **C-D.** SEM photos (Photos: Paul Muir). **E.** *Madrepora damicornis* Esper, 1791. **F.** Specimen identified by Ehrenberg (1834) as *P. verrucosa.* **G.** Corallum of neotype (side view). **H-I**. SEM of neotype.

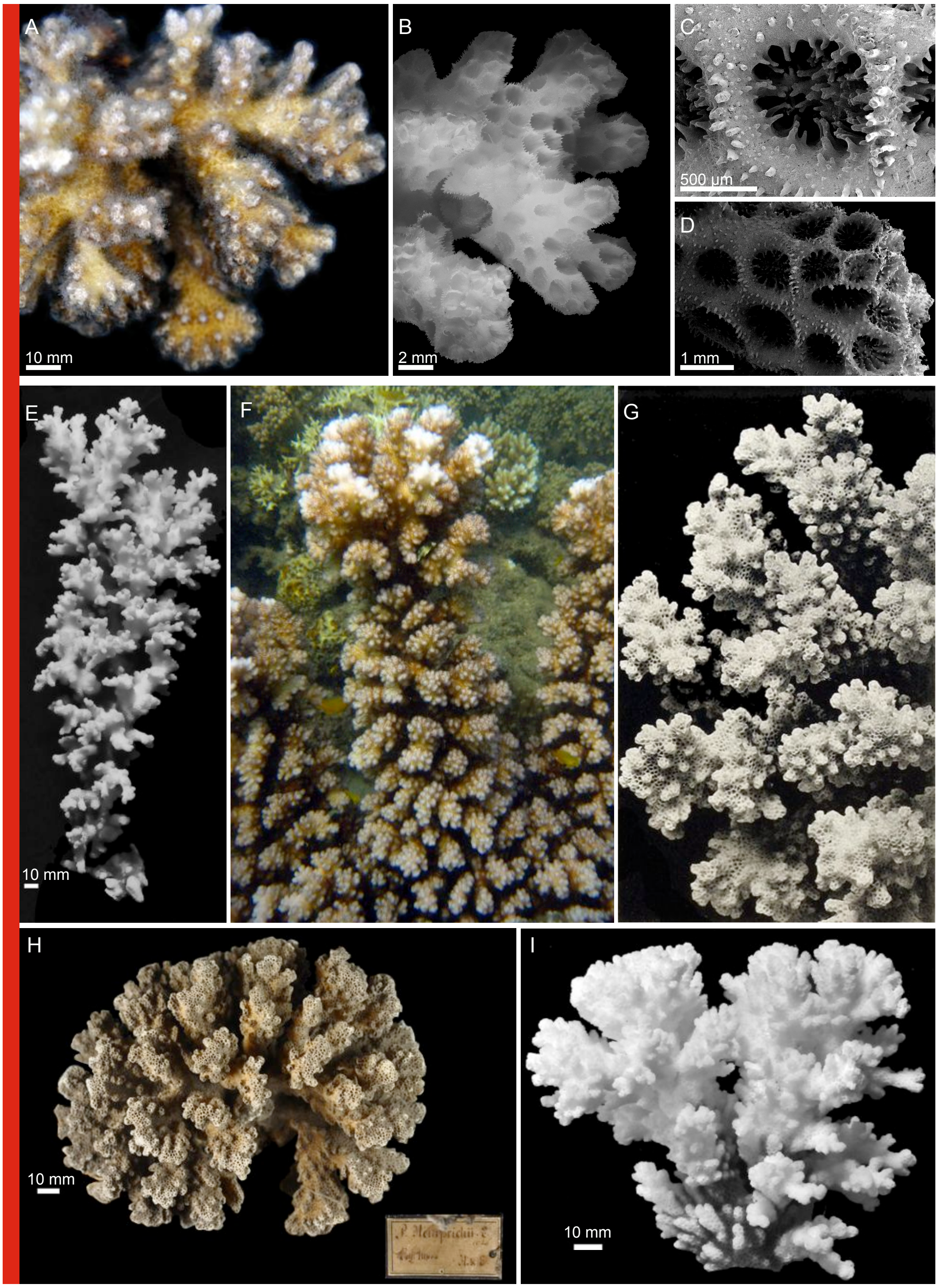

Figure 5.14: *Pocillopora verrucosa* var. *hemprichii.* **A.** *In situ* appearance. **B.** Skeleton of specimen. **C-D.** SEM of specimen (Photos: Paul Muir). **E.** Corallum of elongate "*damicornis*-like" morph. **F.** Colony at reef slope at Orpheus Island. **G.** Holotype of *Pocillopora danae.* **H.** Corallum of holotype of *Pocillopora hemprichii* (side view). **I.** Corallum collected at Lizard Island (side view).

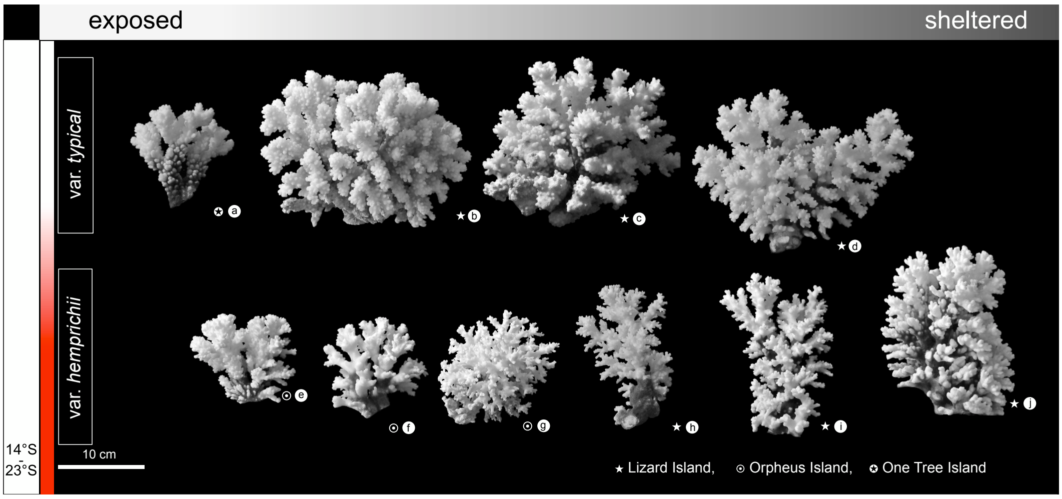

Figure 5.15: Illustration of gross morphology plasticity of the corallum of *Pocillopora verrucosa* in different environments (side views)

teeth and arranged hexamerally in two equally developed cycles. The coenosteum is ornamented sparsely to densely (mostly towards the branch endings) with spinluae, partly elongate in shape.

General description: Two morphological variants can be defined for this taxon. *Pocillopora verrucosa* var. *typical*: Branches subterete, robust, sometimes slightly flattened towards branch tips. Verrucae evenly distributed over corallum, sometimes reduced or missing between branches; verrucae more irregular in height and width than those of *P. meandrina* (see Fig. 5.16 A, I, K). *P. verrucosa* var. *hemprichii*: Branches subterete, robust, often swollen ends and commonly growing in high stalks (>40 cm). Verrucae reduced or absent on main stems, giving it a *P. damicornis* like appearance. However, the branching is more spaced and less cespitose than in *P. damicornis*.

Corallites and coenosteum. As described for neotype.

Colour and pigmentation of the live colony. The colour of this species is usually brownish to pale, rarely pink, with darker pigmentation around oral opening, similar to *P. acuta*.

Remarks. As stressed in Schmidt-Roach et al. (2012a), specimens of *P. verrucosa*, *P. damicornis* Type γ and Tropical Eastern Pacific (TEP) . As stressed in Schmidt-Roach et al. (2012a), specimens of *P. verrucosa*, *P. damicornis* Type γ and Tropical Eastern Pacific (TEP) *P.* cf. *damicornis* are recovered within one mitochondrial clade and share identical haplotypes. Further, the fluent morphological transition between *P. verrucosa* and *P. damicornis*-like morphs from the TEP and of Type γ (reduced to absent verrucae) further strengthens that both present morphotypes of the same taxon. Thus these lineages are here synonymised with *P. verrucosa*. Further, I agree with Sheppard (1987) that *P. danae* Verrill, 1864 presents a synonym of *P. verrucosa*.

Habitat and Biology. The species is often found to arise vertically with monopodial stems (see Fig. 5.15 E, F) and commonly covers larger areas of the reef slope of several square meters. Although the species has been reported to brood larvae, this most likely relates to incorrect identification and the true *P. verrucosa* is a broadcast spawning species (see Schmidt-Roach et al., 2012b).

Distribution. Sequences from public databases indicate a cosmopolitan distribution from the Indian Ocean, Red Sea the Tropical Eastern

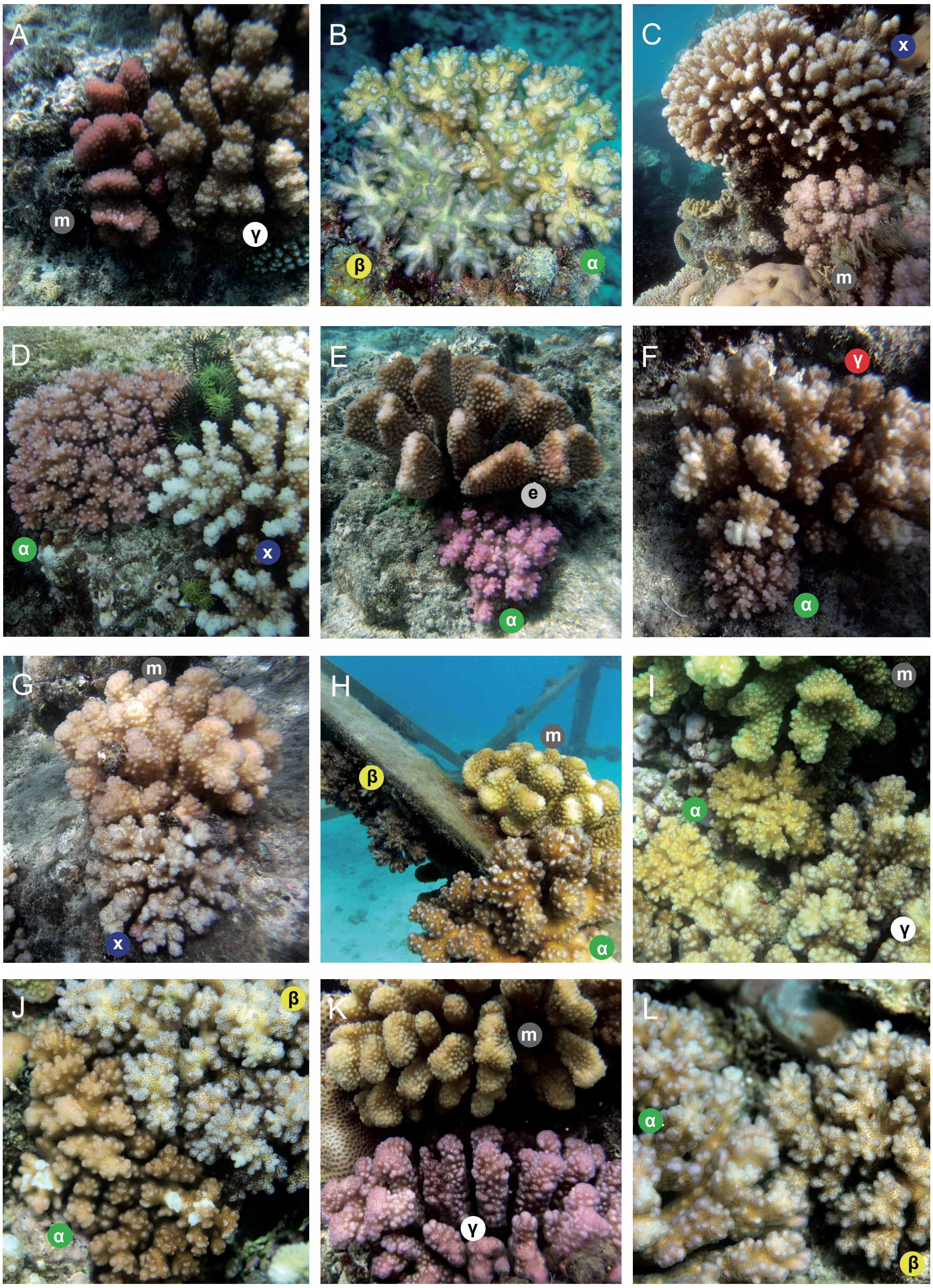

Figure 5.16: Field appearance of taxa in partial sympatry, when growing as mosaic colonies or in approximate distance to each other. **d.** *P. damicornis*, **a.** *P. acuta*, **v.** *P. verrucosa*, **s.** *P. bairdi* sp. nov., **m.** *P. meandrina*, **e.** *P. eydouxi*

Pacific (see Schmidt-Roach et al., 2012a), which matches its distribution based on morphology (see Veron 2000).

5.5.6 *Pocillopora bairdi* sp. nov.

(Fig. 5.17, 5.18)

Holotype: MTQ-G65918 Mermaids Cove, Lizard Island, QLD, Australia (14°38'46.4"S 145°27'19.03"E). 3 m, (Nov 2011) (Coll. S. Schmidt-Roach) (Fig. 5.17 B-E).

Paratypes: 1. MTQ-G65921 Same location as the holotype, 3 m, (Nov 2011) (coll.: S. Schmidt-Roach); 2. MTQ-G65919 same as previous; 3. MTQ-G65920 Trimodal Reef, Lizard Island, QLD, Australia (14°41'56'91S 145°26'52.54) (Fig. 5.17 F).

Other material studied: Lizard Island (1 specimen)

Skeletal characteristics of the holotype. The corallum measures 197mm in length, 147mm in width and 119mm in height. Branches are thin (<1.2 cm in diameter), often flattened towards branch endings. Branch thickness is similar from origin to terminal branch end, which differentiates this species clearly from other *Pocillopora* spp.. Branches growth is directed upward, with sub-branches following this direction shortly after ramification, with spacing between branches of approximately 6mm. Branches are of similar height and densely arranged giving the corallum an overall round shape. Calices are between 0.4–0.6mm in diameter. Verrucae are equally distributed and short (∼1mm), strongly reduced to obsolete at summits.

Corallites and coenosteum of holotype. The columella is absent to styloid, often indicated just by its ornamentation with long spinulae, which may be arranged in a line (similar to *P. verrucosa*); septa are only indicated by irregular, but long (∼100–150μm) spinulate septa teeth and arranged hexamerally in two equally developed cycles. The coenosteum is ornamented sparsely to densely with spinulae. The specimen was pale to light brown when sampled and was found in a mosaic colony in sympatry with *P. damicornis* and *P. meandrina* (Fig. 5.17 G, H).

General description: The species is very distinct in its growth from other taxa of the genus and easy to identify in the field. The corallum is fragile, with evenly sized branches and evenly arranged small verrucae. Overall, all specimens were very similar to the holotype, with one being slightly more elongate and thinner (Fig. 5.17 F).

Colour and pigmentation of the live colony. Mostly pale to light brown, rarely pink.

Remarks. Some specimens were characterised by a unique mitochondrial haplotype in the ORF region; however, others were found to share a common haplotype with *P. verrucosa.* All individuals had a single HSP70 haplotype which was distinct from the haplotypes found in all other species (Fig. S4.2). The lack of clear divergence from *P. verrucosa* in the ORF region may be explained by introgressive hybridisation or incomplete lineage sorting as observed in other taxa of the genus (see Schmidt-Roach et al., 2012a). Nevertheless, the strict divergence from *P. verrucosa* and *Pocillopora damicornis* in the nuclear region and its very distinct and consistent morphology strongly suggests it presents a separate species. Further, it was found to grow close to typical *P. verrucosa* colonies (Fig. 5.16 A, F, D, C, G; photos taken on the reef flat at Mermaids Cove, Lizard Island).

Habitat and Biology. Occurs predominantly in back reef habitats, but was also observed in less exposed habitats of the reef crest. Reproductive mode is unknown.

Distribution. Records are limited to the waters of Lizard Island.

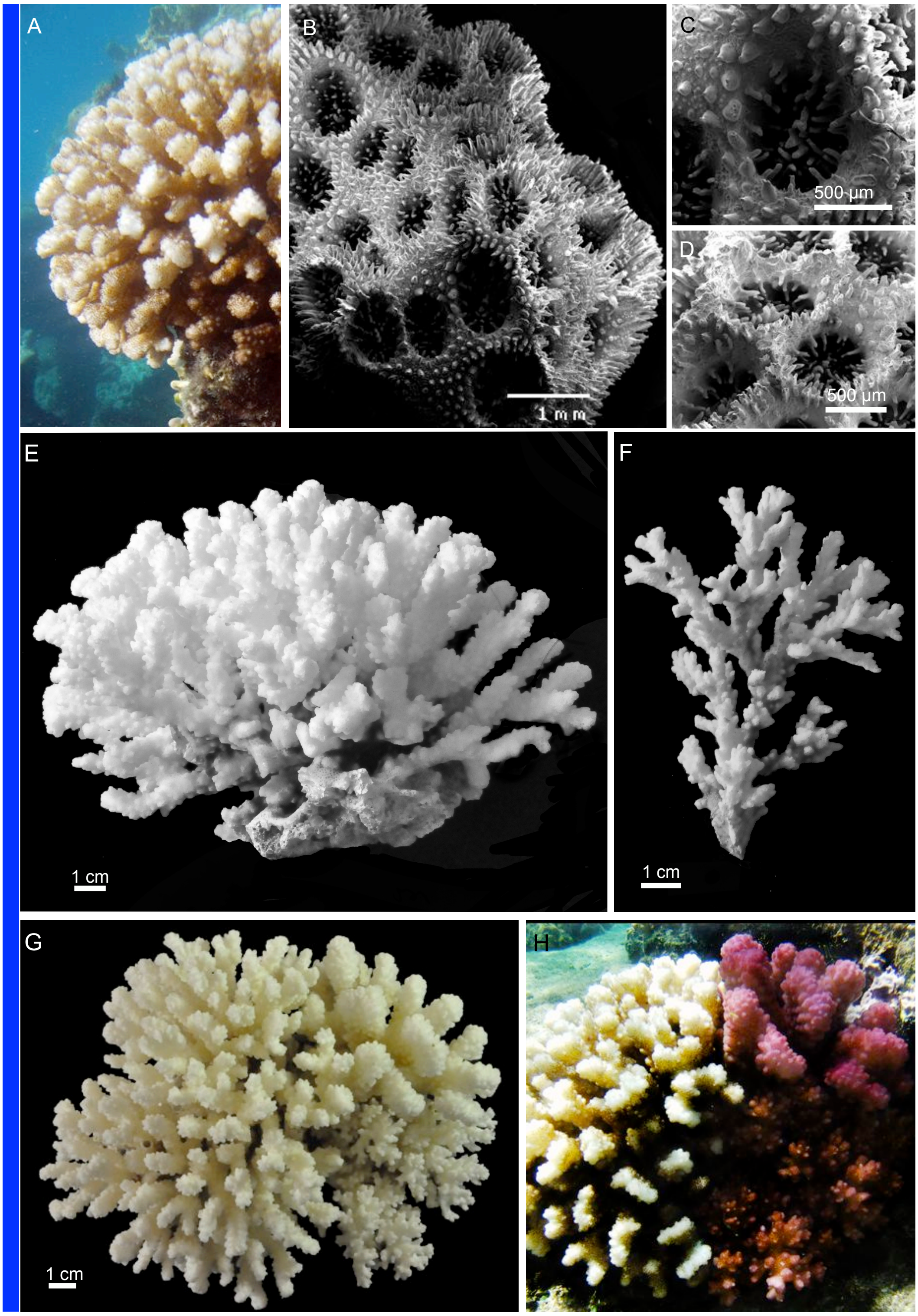

Figure 5.17: *Pocillopora bairdi* **sp. nov.**. **A.** *In situ* appearance. **B.** Skeleton of specimen. *C-D.* SEM of specimen. **E.** Corallum of holotype. **F.** Corallum of paratype MTQ-G65920. **G.** Mosaic colony incl. holotype (left), *P. meandrina* (upper right) and *P. damicornis* (lower right). **H.** Previous colony *in situ*.

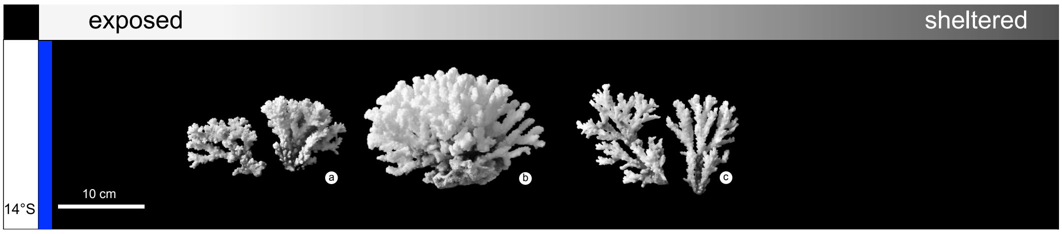

Figure 5.18: Illustration of gross morphology plasticity of the corallum of *Pocillopora bairdi* **sp. nov.** in different environments (side views).

5.5.7 *Pocillopora eydouxi* Edwards & Haime, 1860

(Fig. 5.19, 5.20)

Synonymy

Pocillopora grandis Dana, 1846 p. 533
Pocillopora elongata Dana, 1846 p. 531
Pocillopora eydouxi Edwards & Haime, 1860
?Pocillopora coronate Gardiner, 1897 p. 949
Pocillopora rugosa Gardiner, 1897 p. 950

Taxonomic history. *P. eydouxi* was described by Edwards & Haime (1860). Although it presents a junior synonym of *P. grandis* Dana, 1846 and *P. elongata* Dana, 1846, these names are suppressed due to the common use of the name *P. eydouxi* for specimens of this taxon (Veron & Pichon, 1976).

Material studied. *MTQ-samples:* G33435, G33432, G33428 Tijou Reef, QLD, Australia (13°10'S 143°57'E); G33430, G33434 Great Detached Reef, QLD, (11°48'S 144°03'E); 33429 Brisk Island, QLD, Australia (18°47'S 146°42E). G33433 Bowl Reef, QLD (18°31'S 147°32'E); Great Detached Reef, QLD (11°42'S 144°00'E); G52299 South China Sea, Scarborough Reef (15°07'N 117°51'E); G50761 Seribu Islands, Indonesia (05°35'S 106°32'E). *Further material:* Lizard Island (3 specimens).

Corallum: The corallum is ramose, verrucate, branching varies from meandering-lamellar, broad-ended to cylindrical (Fig. 5.20). In adult specimens, branches are more robust then in other species (2–4 cm thick). Veron & Pichon (1976) observed colonies up to 95 cm high.

Corallites and coenosteum: The species is distinguished from *P. meandrina* by its styliform columella, with one to three distinct stylae (even within one colony, Fig. 5.19 C) originating from a diagonally arranged, bridge-like columella. Calices are 0.6–1mm in diameter. Septa are hexamerally arranged in two cycles and weakly developed, often only indicated by short (100μm) septa teeth. The second cycle may be weakly developed. The 1st and 4th septa of the first cycle eventually merge into the bridge like columella connecting both sides of the calice. The coenosteum is ornamented with short spinulae.

Colour and pigmentation of the live colony. Evenly pigmented brown to pink.

Remarks. Although this species shares identical mitochondrial lineages with *P. eydouxi*, Flot et al. (2008) found some indication of divergence in the nuclear DNA regions. The most striking difference between this species and *P. meandrina* is however the columella development. All *P. eydouxi* colonies where characterised by a styloid columella development, a feature Edwards & Haime (1860) stressed in the illustration of the type specimen. In contrast, columellae of *P. meandrina* were mostly oval convex, rarely reduced, if styloid with a significant thicker stylus (Fig 5.3 C) *P. eydouxi* (Fig. 5.3 B, C).

Habitat and Biology. This species was found to occur predominantly in exposed to moderate environments.

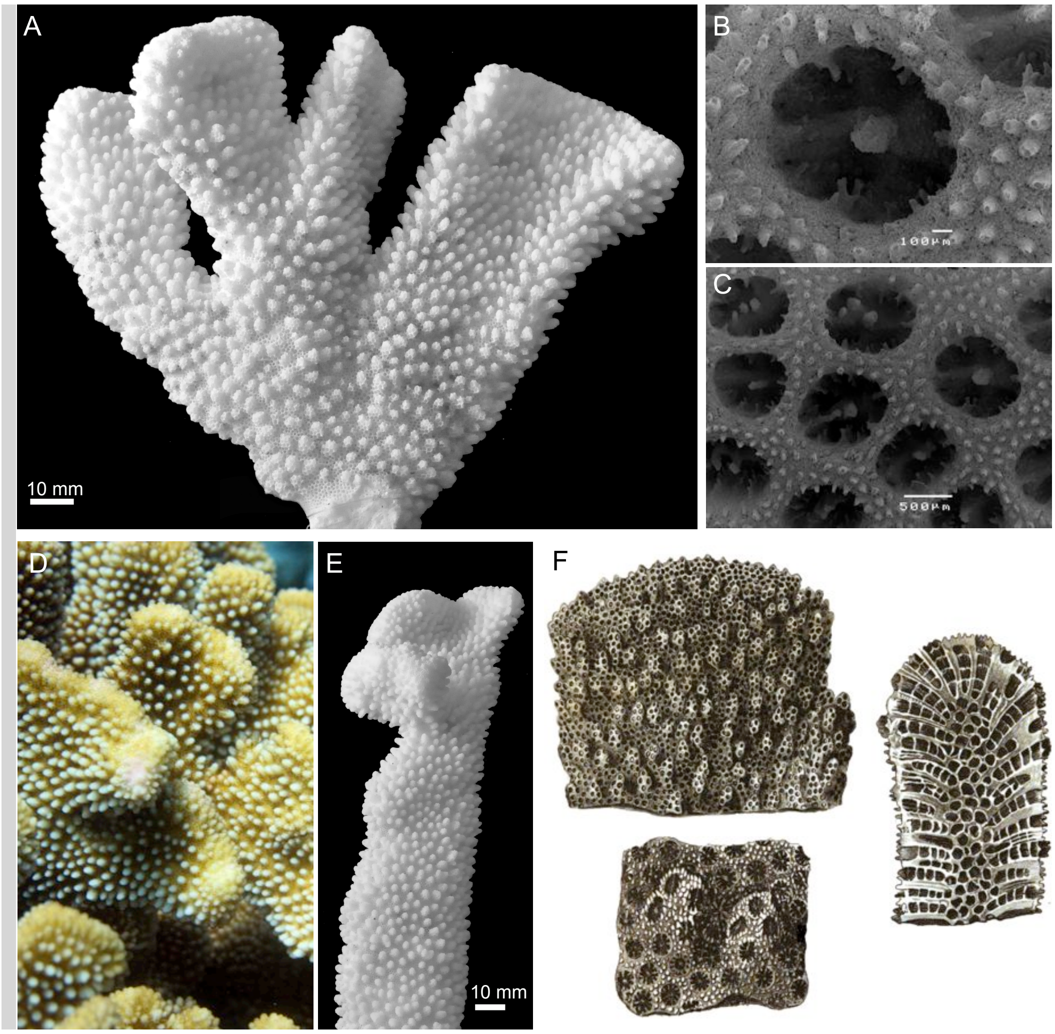

Figure 5.19: *Pocillopora eydouxi*. **A.** Skeleton of specimen (side view). **B-C** SEM of specimen. **D.** *In situ* appearance. **E.** Corallum of cylindrical morph. **F.** Illustration of holotype by Edwards & Haime (1860).

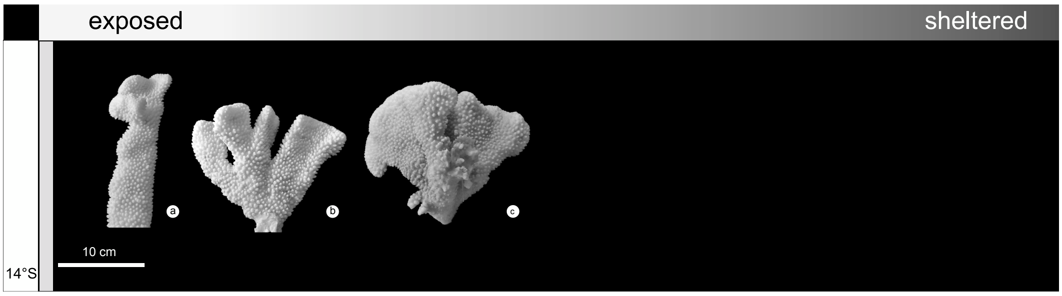

Figure 5.20: Illustration of gross morphology plasticity of the corallum of *Pocillopora eydouxi* in different environments (side views).

Distribution. This species has a cosmopolitan distribution from the Tropical Eastern Pacific to the Indian Ocean and the Red Sea (see Veron 2000) (Fig. 5.5).

5.5.8 *Pocillopora meandrina* Dana, 1846

(Fig. 5.21, 5.22)

Synonymy

Pocillopora meandrina Dana, 1846 p. 533
Pocillopora nobilis Verill, 1864 p. 59

Taxonomic history. The species was described by Dana (1846). Vaughan (1918) notes that *P. meandrina* is closely related to *P. elegans* and *P. verrucosa*, and that it is probable that they are all variants of one species. Veron & Pichon (1976) first synonymised the species under *P. verrucosa*, before describing it as a separate species.

Material studied. MTQ samples: G33035 Mellish Reef Lagoon, QLD, Australia (0-4 m) (19°09S 150°9E); G33036 same as before (5-15 m); G33037 same as before (0-4 m); G52311 South China Sea, Pratas Reef (20°35N 116°46E). *Further material*: Orpheus Island (4 specimens), Lizard Island (3 specimens), Davies Reef (2 specimen).

Corallum: Cespitose, very neatly verrucose, summits often naked. Vaughan (1907) differentiated between two variants within the same species. *P. meandrina* var. *typical*: Branches mostly lamellar and sinuous, evenly verrucate, summits mostly naked (Fig. 5.21 A, B). *P. meandrina* var. *nobilis*: very similar to *P. verrucosa* var. *typical* (Fig. 5.10 A, I, K) in gross morphology, however, verrucae are more neatly and equally arranged and branches are more flattened, forming meanders towards the tips (5.21 E, F).

Corallites and coenosteum: The columella and septa development of this species is very variable (Fig. 5.3 g, Fig. 5.21 C, D). The columella is oval-convex to styloid, rarely obsolete, similar to *P. eydouxi* the columella is diagonal in the calice, but consistently more predominant than that of *P. eydouxi*. Calices are 0.5–1.6mm in diameter. Intra colony variation in the columellae is high, ranging from oval-convex to thick (>200μm) styloid, but characterised by a fine, short spinulate ornamentation. Septa are hexamerally arranged in two cycles and vary in their development from almost reduced to well indicated by long spinulae. Similar to *P. eydouxi* the second septal cycle may be slightly less developed than the first. The coenosteum is ornamented with short spinulae.

Colour and pigmentation of the live colony. Evenly coloured (yellow, brown, pink, blue, or green).

Remarks: Separation between *P. meandrina* var. *nobilis* and *P. verrucosa* has challenged previous studies (e.g. Vaughan, 1918; Veron & Pichon, 1976) due to the gross morphological similarity. In contrast to the morphology, genetically *P. verrucosa* and *P. meandrina* are very distinct genetically and *P. meandrina* has only limited genetic divergence from *P. eydouxi* in the investigated regions.

Habitat and Biology. Reef crest, back reef and reef slope habitats, predominantly in exposed habitats.

Distribution. This species has a cosmopolitan distribution from the Tropical Eastern Pacific to the Indian Ocean (see Veron 2000). However, further research is needed to identify its actual range (Fig. 5.5).

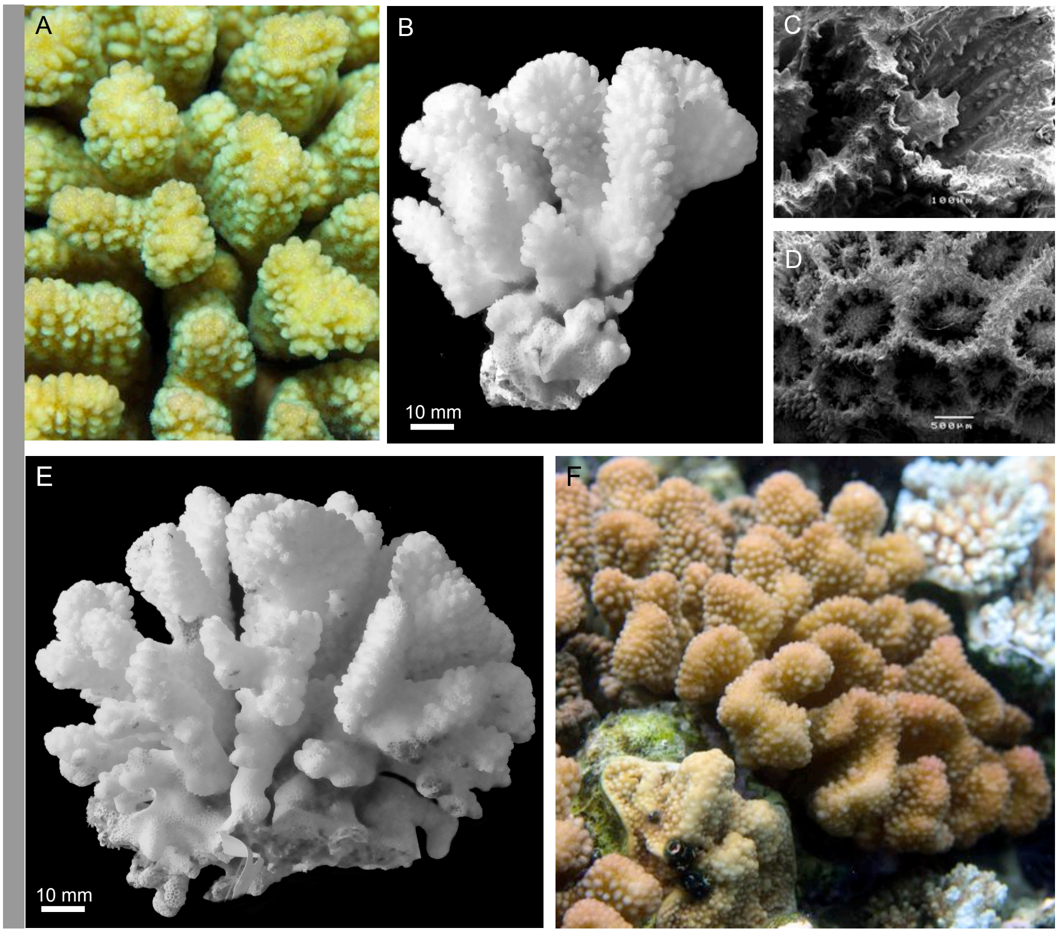

Figure 5.21: *Pocillopora meandrina.* **A.** Field appearance of *P. meandrina* var. *nobilis.* **B.** Skeleton of previous variation. **C-D.** SEM of previous specimen. **E.** Corallum of *P. meandrina* (typical) (side view). D. *In situ* appearance of previous variation.

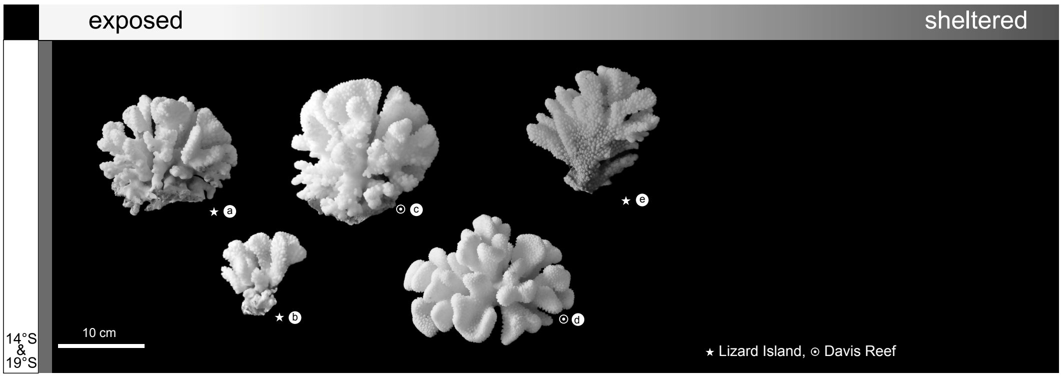

Figure 5.22: Illustration of gross morphology plasticity of the corallum of *Pocillopora meandrina* in different environments (side views).

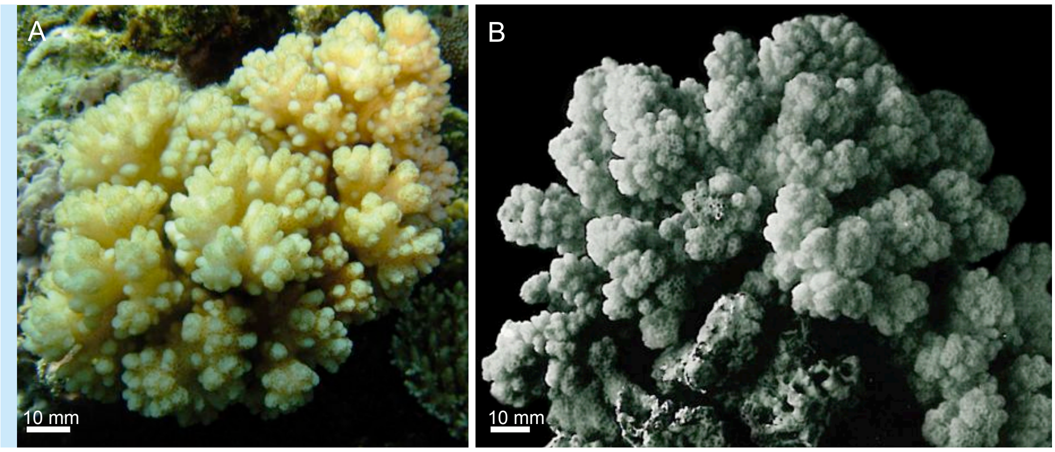

Figure 5.23: *Pocillopora* cf. *brevicornis*. **A.** Field appearance. **B.** Specimen collected and photographed by Hoffmeister (1925).

5.5.9 *Pocillopora* cf. *brevicornis* Lamarck, 1816

(Fig. 5.23)

Synonymy

Pocillopora brevicornis Lamarck, 1816 p. 275

Taxonomic history. Lamarck (1816) described *Pocillopora brevicornis*. Hoffmeister (1925) later added the subspecies *Pocillopora brevicornis setchelli*. The taxon was later synonymised under *P. damicornis* (Veron & Pichon, 1976).

Material and locations studied. Great Detached Reef (1 specimen) (11°45'0"S 144°1'1E)

Corallum. Branches short, compressed, thick, even-topped, and crowded with irregular but short sub-branching/verrucae.

Colour and pigmentation of the live colony. Evenly pale to yellow.

Remarks. Unfortunately access was limited to a small fragment of one specimen, thus limiting a formal resurrection of this taxon. However, photographs of the specimen sampled correlate well with the description for *P. brevicornis*. Further, its genetic divergence strongly indicates that it is a distinct species. Future investigations are needed to confirm the validity of this taxon.

Distribution: At this point in time is only confirmed location is the Northern Great Barrier (Fig. 5.5), however, it may have been missed in other locations due to is rarity.

Table 5.2: List of samples collected in this study

MTQ-number	**Code**	**Species**	**ORF** see Fig. 5.3	**Environment**	**Latitude**	**Longitude**	**Depth**	**Figure**	**Morpho-metrics**
G66090	LZI 5	*P. acuta*	β	lagoon, crest of boulder	14°41’14”S	145°27’57”E	7 m	n/a	yes
G66129	LZI 8	*P. acuta*	β	boulder flat	14°41’14”S	145°27’57”E	1 m	5.10 e	yes
G66089	LZI 9	*P. acuta*	β	boulder crest	14°41’14”S	145°27’57”E	4 m	5.10 i	yes
G66112	LZI 10	*P. acuta*	β	lagoon bottom	14°41’14”S	145°27’57”E	3 m	5.10 h, 5.9 I	yes
G66112	LZI 11	*P. acuta*	n/a	lagoon, crest of boulder	14°41’14”S	145°27’57”E	5 m	5.10 j	yes
G66137	LZI 30b	*P. acuta*	β	reef crest exposed	14°41’56”S	145°26’52”E	3 m	n/a	n/a
G66135	LZI 31b	*P. acuta*	β	reef crest exposed	14°41’56”S	145°26’52”E	3 m	n/a	yes
G66127	LZI 34b	*P. acuta*	β	reef crest exposed	14°41’56”S	145°26’52”E	5 m	5.10 g	yes
G66133	LZI 36	*P. acuta*	β	lagoon bottom	14°41’14”S	145°28’8”E	4 m	5.10 d	yes
G66130	LZI 41	*P. acuta*	β	reef crest shallow	14°38’46”S	145°27’19”E	2 m	5.10 a	yes
G66132	LZI 44	*P. acuta*	n/a	crest towards exposed site	14°41’56”S	145°26’52”E	4 m	5.10 f	yes
G66105	OPI 4x	*P. acuta*	β	back reef, moderate	18°33’13”S	146°29’18”E	3 m	5.10 c	yes
G66106	OPI F2	*P. acuta*	β	back reef, moderate	18°33’13”S	146°29’18”E	3 m	5.10 b	yes
n/a	OPI 38	*P. acuta*	β	back reef, sheltered	18°33’13”S	146°29’18”E	6 m	5.9 A-D	n/a
G65423	SOL 1	*P. aliciae*	δ	rocky habitat, exposed	30°12’0”S	153°15’27”E	8 m	5.12 a	yes
G65424	SOL 2	*P. aliciae*	δ	rocky habitat, exposed	30°12’0”S	153°15’27”E	12 m	5.12 b	yes
G66108	SOL 3	*P. aliciae*	δ	rocky habitat, exposed	30°12’0”S	153°15’27”E	8 m	n/a	yes
G66122	LZI 18	*P. damicornis*	α	crest, on canyon	14°41’56”S	145°26’52”E	3 m	n/a	yes
G66109	LZI 19	*P. damicornis*	α	bottom, next to Type C	14°41’56”S	145°26’52”E	5 m	5.7 e	yes
G66095	LZI 20	*P. damicornis*	α	crest, moderate	14°41’56”S	145°26’52”E	4 m	5.7 g	yes
G66125	LZI 24	*P. damicornis*	α	lagoon bottom	14°41’14”S	145°28’8”E	4 m	n/a	yes
G66916	LZI 27	*P. damicornis*	α	lagoon bottom	14°38’46”S	145°27’19”E	3 m	n/a	yes
G66126	LZI 30a	*P. damicornis*	α	reef crest exposed	14°41’56”S	145°26’52”E	2 m	5.7 c	yes
G66136	LZI 31a	*P. damicornis*	α	reef crest exposed	14°41’56”S	145°26’52”E	3 m	5.7 d	yes
G66134	LZI 32	*P. damicornis*	n/a	reef slope, sheltered	14°41’47”S	145°28’28”E	4 m	5.7 i	n/a
G66127	LZI 34a	*P. damicornis*	α	Reef slope, sheltered	14°41’56”S	145°26’52”E	5 m	5.7 h	yes
G66101	LZI 35	*P. damicornis*	α	reef crest exposed	14°41’5”S	145°28’17”E	3 m	n/a	n/a
G66102	LZI 38	*P. damicornis*	α	reef crest exposed	14°41’56”S	145°26’52”E	3 m	5.7 a	yes
G66107	LZI 40	*P. damicornis*	α	reef crest moderate-exposed	14°41’56”S	145°26’52”E	3 m	5.7 f	yes
G66131	LZI 41	*P. damicornis*	α	reef crest shallow	14°38’46”S	145°27’19”E	3 m	5.7 b	yes
n/a	OTI G	*P. damicornis*	α	sheltered, lagoon back reef	23°30’30”S	152°05’30”E	2 m	n/a	n/a
n/a	OTI H	*P. damicornis*	α	sheltered, lagoon back reef	23°30’30”S	152°05’30”E	2 m	n/a	n/a
G66110	OTI PA2	*P. damicornis*	α	reef flat, moderate	23°30’30”S	152°05’30”E	3 m	n/a	yes
G66138	OTI PA1	*P. damicornis*	α	reef flat, moderate	23°30’30”S	152°05’30”E	3 m	n/a	n/a
G66098	OTI PA3	*P. damicornis*	α	lagoon, moderate-sheltered	23°30 30”S	152°05’30”E	5 m	5.7 n	yes
G66097	OTI PA4	*P. damicornis*	α	lagoon, exposed	23°30’30”S	152°05 30”E	2 m	5.7 k	yes
G66099	OTI PA5	*P. damicornis*	α	lagoon, low	23°30’30”S	152°05’30”E	3 m	5.7 o	yes

G66103	OTI PA6	*P. damicornis*	α	reef crest, exposed	23°30’30”S	152°05’30”E	2 m	5.7 l	yes
G66124	OTI PA7	*P. damicornis*	α	reef slope, moderate	23°30’30”S	152°05’30”E	12 m	n/a	yes
G66123	OTI PA8	*P. damicornis*	α	reef slope, moderate	23°30’30”S	152°05’30”E	2 m	5.7 j	yes
G66100	OTI.lag	*P.damicornis*	α	lagoon, sheltered	23°30’30”S	152°05’30”E	1 m	5.7 p	yes
n/a	OTI PA9	*P.damicornis*	n/a	reef slope, moderate	23°30’30”S	152°05’30”E	9 m	5.7 m	yes
G66091	R1	*P. damicornis*	α	rocky habitat, exposed	32°0’16”S	115°30’4”E	2 m	5.7 t, 5.6 G	yes
G66093	R2	*P. damicornis*	α	rocky habitat, exposed	32°0’16”S	115°30’4”E	2 m	5.7 q	yes
G66094	R3	*P. damicornis*	α	rocky habitat, exposed	32°0’16”S	115°30’4”E	2 m	5.7 r	yes
G66121	R4	*P. damicornis*	α	rocky habitat, exposed	32°0’16”S	115°30’4”E	2 m	5.7 s	yes
G66090	R5	*P. damicornis*	α	rocky habitat, exposed	32°0’16”S	115°30’4”E	2 m	5.7 u, 5.6 F	yes
G66092	R6	*P. damicornis*	α	rocky habitat, exposed	32°0’16”S	115°30’4”E	2 m	n/a	yes
G66120	LZI 1	*P. eydouxi*	n/a	reef crest exposed	14°41’56”S	145°26’52”E	2 m	5.20 c	yes
G66118	LZI 2	*P. eydouxi*	m	lagoon, relative exposed	14°41’14”S	145°27’57”E	2 m	5.20 b	yes
G66117	LZI 3	*P. meandrina*	n/a	reef crest exposed	14°41’56”S	145°26’52”E	3 m	5.22 c	yes
G66113	LZI 4	*P. meandrina*	m	back reef, moderate	14°41’14”S	145°28’8”E	2 m	5.22 e	yes
G66917	LZI 27	*P. meandrina*	m	lagoon bottom	14°38’46”S	145°27’19”E	3 m	5.22 b	yes
G66115	DAV 1	*P. meandrina*	n/a	reef flat, moderate-exposed	18°49’6”S	147°38’58E	1 m	5.22 a	n/a
G66116	DAV 2	*P. meandrina*	n/a	reef flat, moderate-exposed	18°49’6”S	147°38’58E	3 m	5.22 d	n/a
G66119	LZI 43	*P. eydouxi*	m	reef crest exposed	14°41’5”S	145°28’17”E	3 m	5.20 a	yes
G66143	LZI 6	*P. verrucosa*	γ	boulder crest exposed	14°41’14”S	145°27’57”E	5 m	5.15 h	yes
G66147	LZI 7	*P. verrucosa*	γ	lagoon, boulder crest exposed	14°41’14”S	145°27’57”E	4 m	5.15 j	yes
G65923	LZI 12	*P. verrucosa*	γ	boulder crest	14°41’14”S	145°27’57”E	2 m	5.15 c	yes
n/a	LZI 13	*P. verrucosa*	n/a	reef crest exposed	14°41’14”S	145°27’57”E	4 m	n/a	n/a
G66114	LZI 14	*P. verrucosa*	n/a	bottom	14°41’14”S	145°27’57”E	6 m	5.15 b	n/a
n/a	LZI 15	*P. verrucosa*	n/a	lagoon	14°41’14”S	145°28’8”E	3 m	n/a	no
G66144	LZI 25	*P. verrucosa*	γ	lagoon bottom	14°41’14”S	145°28’8”E	5 m	5.15 e	yes
n/a	LZI 26	*P. verrucosa*	γ	lagoon bottom	14°41’14”S	145°28’8”E	4 m	n/a	No
G66141	LZI 28	*P. verrucosa*	γ	reef crest exposed	14°39’47”S	145°28’29”E	6 m	n/a	yes
G66148	LZI 37	*P. verrucosa*	γ	bottom	14°41’56”S	145°26’52”E	6 m	n/a	yes
G66145	LZI 39	*P. verrucosa*	γ	lagoon bottom	14°41’14”S	145°28’8”E	5 m	5.15 i	yes
G66142	OPI L2	*P. verrucosa*	γ	reef slope, sheltered	18°33’13”S	146°29’18”E	6 m	5.15 d	n/a
G66149	OPI K2	*P. verrucosa*	γ	reef slope, sheltered	18°33’13”S	146°29’18”E	4 m	n/a	yes
G66139	OPI D3	*P. verrucosa*	γ	reef flat, moderate-exposed	18°33’13”S	146°29’18”E	2 m	5.15 f	n/a
G66140	OPI J	*P. verrucosa*	γ	reef flat, moderate	18°33’13”S	146°29’18”E	3 m	5.15 g	n/a
G66146	OTI PC3	*P. verrucosa*	γ	reef slope exposed	23°30’30 S	152°05’30”E	5 m	5.15 a	yes
n/a	GD 1	?*P.* cf. *brevicornis*	ϵ	reef slope	11°45’0” S	144°1’1” E	2-8 m	5.23 A	n/a
G65921	LZI 21	*P. bairdi* sp. nov	γ	reef crest shallow	14°38’46”S	145°27’19”E	2 m	n/a	yes
G66104	LZI 22	*P. bairdi* sp. nov	γ	reef crest shallow	14°38’46”S	145°27’19”E	3 m	5.18 a	yes

G66920	LZI 23	*P. bairdi* sp. nov	x	reef crest, in canyon	14°38’46”S	145°27’19”E	3 m	n/a	yes
G66919	LZI 33	*P. bairdi* sp. nov	x	reef crest exposed	14°41’56”S	145°26’52”E	3 m	5.18 c	yes
G65918	LZI 27	*P. bairdi* sp. nov	γ	lagoon bottom	14°38’46”S	145°27’19”E	3m	5.18 b	yes

CHAPTER
SIX

THE RELATIVE IMPORTANCE OF INBREEDING AND OUTBREEDING IN CORALS

6.1
INTRODUCTION

In an evolutionary context, fitness is linked to success in passing genes to the next generation - hence mechanisms that ensure successful reproduction under a range of environmental conditions are likely to be adaptive. Sexual reproduction requires sperm and eggs to be united with those of possible mating partners, a challenge especially for sessile organisms that cannot move to find a suitable mate. In terrestrial systems, many sessile organisms have evolved complex symbioses with pollinators in order to guarantee gamete dispersal and pollination/fertilisation. Many of these symbioses are species-specific (Darwin, 1876), thus promoting an efficient gamete dispersal and fertilisation system. In stark contrast to the terrestrial world, in marine environments pollinators are absent and fertilisation success of sessile organisms is dependent on the passive dispersal and often chance encounters of sperm and eggs in the water column (e.g. Harrison et al., 1984). Under these circumstances, factors such as the distance between mating partners becomes important because gamete concentration decreases with distance from the spawning adult and can result in lower fertilisation rates (e.g. Levitan et al., 1992; Oliver & Babcock, 1992; Sewell & Levitan, 1992; Levitan & Petersen, 1995). As a result, a huge diversity of different reproductive strategies has evolved in sessile marine species, including plants and animals, to facilitate gamete mixing and maximise fertilisation success.

Reproduction in marine organism generally falls into two broad categories; broadcast spawning species release their gametes to be fertilised in the water column, whereas brooding species are characterised by sperm release but with internal fertilisation and embryogenesis. While brooding species may maintain unfertilised eggs internally for days or even weeks thus maximising the chances of eggs being fertilised by drifting sperm, species that spawn gametes for external fertilisation depend highly on synchronous release (Pennington, 1985; Harrison & Wallace, 1990). Synchronous mass spawning (linked to environmental cues e.g. lunar phase, temperature or chemical cues) increases chances of egg-sperm encounters and reduces predation leading to a better survival of the offspring (Beach et al., 1975; Babcock et al., 1986; Harrison & Wallace, 1990). In addition, other mechanisms to concentrate gametes and increase fertilisation in spawning species have evolved. In many corals gametes are released in positively buoyant egg-sperm bundles, which concentrate gametes at the water's surface (Babcock et al., 1986). More mobile benthic species such as starfish can engage in activities such as spawning aggregations (e.g. Babcock et al., 1992) and pseudo-copulation (e.g. Slattery & Bosch, 1993; Hamel & Mercier, 1995; Keesing et al., 2011) to facilitate fertilisation. Furthermore, during multi-species spawning events intra-specific fertilisation is likely promoted by chemical sperm attractants and pheromones such as observed in abalone (Riffell et al., 2002; Krug et al., 2009) as well as inter-specific gamete incompatibility (e.g. Willis et al., 1997).

Most spawning coral species are hermaphrodites (e.g. Harrison & Wallace, 1990; Baird et al., 2009; Harrison, 2011), a strategy that potentially enhances fertilisation success considering that in hermaphrodites any conspecific is a potential mating partner. As sperm seems to be limiting in marine environments, fertilisation success will most likely be population density dependent (Levitan & Petersen, 1995). Thus, larvae of sessile marine organisms often recruit near conspecifics (and in their natal habitat e.g. Maier et al., 2005) in part to increase mating success (see Carlon, 1999). However, while philopatric recruitment may promote fertilisation, it may lead to considerable inbreeding (Grosberg, 1987), resulting in the loss of genetic diversity and inbreeding depression (Charlesworth & Charlesworth, 1987), which can cause substantial fitness reductions compared to outbred populations (Keller & Waller, 2004). However, in the absence of non-self sperm, self-fertilisation may be an important strategy to ensure fertilisation success (Heyward & Babcock, 1986; Miller & Mundy, 2005) and indeed inbreeding, including self-fertilisation, has been argued to be advantageous in the marine environment (Knowlton & Jackson, 1993). High selfing-rates in corals suggest that selfing may be an important mode of reproduction in some hermaphroditic marine invertebrates (Stoddart et al., 1988; Brazeau et al., 1998). However, although selfing may guarantee fertilisation, larvae originating from self-fertilisation were found to have lower survival rates when exposed to temperature stress than those from outcrossed matings (Woolsey, 2012). Furthermore, self-compatibility seems to be rather rare and does not occur in many species (e.g. Miller & Babcock, 1997; Willis et al., 1997).

Much of what we know about reproductive relationships, including the ability of species to self-fertilise stems from *ex situ* fertilisation experiments, and relies on the assumption that laboratory trials adequately reflect processes that are occurring in natural environments, although this has never been tested empirically. For example, in laboratory experiments the reef coral *Goniatsrea favulus* readily self-fertilises (Babcock et al., 1986), suggesting self-fertilisation may be an important life-history strategy in this species (Stoddart et al., 1988), although such fertilisation trials invariable only include a single sperm source and hence do not address whether selfing might occur in the presence of non-self sperm. Self-compatibility may also contribute to the high (>94%) *in situ* fertilisation rates recorded for *G. favulus* (Miller & Mundy, 2005), although a recent genetic study at One Tree Island (OTI) found limited evidence of inbreeding and inbreeding depression in populations (Miller & Ayre, 2008a). Thus, the high fertilisation success may actually be linked to high population densities and low water levels at the time of spawning, despite the ability of *G. favulus* to self-fertilise (Miller & Mundy, 2005).

Goniastrea favulus releases gametes freely and has sticky, negatively buoyant eggs that remain attached to the maternal colony through fertilisation (Quinn & Kojis, 1981; Heyward & Babcock, 1986; Miller & Mundy, 2005), it is an ideal species for paternity experiments as the maternal colony is easily identified. Sperm are released subsequent to the release of eggs (Quinn & Kojis, 1981) (Fig. 1.3). Following spawning, the egg clumps gradually drift from the surface of the colony and sink, eventually adhering to the substrate in relatively close proximity to the parent colony (Quinn & Kojis, 1981). In this study I explore the importance of self-fertilisation in the common reef coral *Goniastrea favulus* by employing newly developed microsatellite markers to assess paternity of larvae fertilised in the laboratory and under natural conditions on the reef. Further, I test if *G. favulus* favours non-self over self-fertilisation in situations of sperm choice. Furthermore, direct comparisons between *in situ* and *ex situ* selfing rates in *G. favulus* provide a unique perspective on the limitations of laboratory fertilisation trials for understanding natural reproductive processes in the marine environment.

6.2

MATERIAL AND METHODS

6.2.1 *Ex situ* self-fertilisation trials

Twenty *Goniastrea favulus* colonies were collected from the reef flat at OTI Reef (23°30S, 152°05E) one day before the annual spawning in November 2011. Colonies were randomly assigned to tanks and maintained with flow-though seawater prior to spawning. To keep the experimental systems simple and guarantee paternity assignment, tanks contained either two colonies (in a total volume of 50L) or four colonies (in a total water volume of 180L). Just prior to 3pm on the 5th day following full moon (the predicted time of spawning of *G. favulus* at OTI Reef; Miller & Mundy, 2005) flow-through water was turned off and colonies allowed to spawn and gametes to mix naturally. The times of egg and sperm release of all colonies were recorded. Eggs were collected from each colony approximately 1–2 hours after egg release using a Pasteur pipette to minimise water turbulence and transferred to glass vials held at ambient water temperature to allow embryogenesis. After 48hrs individual planulae where then preserved in <40μl 90% ethanol for later genetic analysis. In addition, a tissue sample from each spawning colony was preserved in 90% ethanol.

6.2.2 *In situ* self-fertilisation trials

During the annual spawning of at OTI Reef in November 2002 eggs fertilised *in situ* were collected in order to determine the level of self-fertilisation that occurs in *G. favulus* under natural conditions on the reef. Just prior to the predicted spawning, eight *G. favulus* colonies within two fixed plots on the southern reef flat were targeted (Miller & Ayre, 2008a). Spawning times were noted, and eggs collected from each colony approximately 1hr following release. Eggs were rinsed in a 105μM plankton mesh sieve with sperm-free water to remove excess sperm that may subsequently fertilise eggs and bias results. Larvae were allowed to develop for 48hrs at ambient temperature in 25mL volumes of sperm-free seawater to complete embryogenesis and were then frozen individually in liquid nitrogen. Tissue samples of tagged adult colonies were also collected for genotyping (see Miller & Ayre, 2008a).

6.2.3 Development of Microsatellite DNA markers

Although some microsatellite markers already exist for *G. favulus* (Miller & Howard, 2004) I found these unreliable for genotyping larvae. Consequently I developed additional microsatellite markers for use in this study. For this, DNA was extracted using a Qiagen DNeasy Tissue and Blood Extraction Kit from ten larvae to ensure sufficient DNA was available for marker development and to avoid Symbiodinium in adult tissue that would confound the marker development.

Partial genome (shot-gun) sequencing on a 454 platform was performed to identify microsatellite sequences within the genome of *G. favulus* in a combined multi-species run at the Australian Genomic Research Facility (AGRF; Brisbane, Australia) coordinated by Dr. M. Gardner (School of Biological Sciences, Flinders University) following protocols in Gardner et al. (2011). Generated sequence information was analysed and mined for microsatellite regions using ODDv2 (Meglécz et al., 2010). One-eighth of a plate of shot-gun sequencing yielded >1700 microsatellite regions with unique flanking regions. Unlabelled primer pairs flanking 29 microsatellites were selected following the guidelines of Gardner et al. (2011). Tetra- or penta-nucleotide repeats were given preference in order to support reliable scoring (Gardner et al., 2011).

Unlabelled primers were used to assess the level of polymorphism at each of the 29 loci. A sample containing the pooled DNA of 20 individuals was PCR-amplified for each primer pair. The PCR product was then screened using gel-electrophoresis (4% Agarose gel, 80V, 3hrs) for products showing multiple bands matching the targeted fragment range. Twelve promising polymorphic loci were selected and each amplified using fluorescence-labeled forward primers in four randomly selected individuals and one sample containing the pooled DNA of 20 individuals. The latter was used to estimate the allelic range of each region. All PCR products were sent to Macrogen for genotyping (Genescan ABI3730XL). This procedure resulted in seven bioinformative microsatellite marker pairs with tetra- or penta-repeats (Table 6.1).

6.2.4 DNA extraction, amplification and genotyping:

DNA of spawning adults from the tank experiment was extracted using a QiagenDNeasy Tissue and Blood Extraction Kit and the DNA of their larvae was extracted using a protocol optimised for single-fly DNA preparation for PCR (Gloor et al.,1993). DNA of larvae collected *in situ* and frozen to -80°C were extracted using a QiagenQIAamp DNA Micro Kit according to manufacturers protocols. DNA was amplified in a combination of multiplex and single-plex PCR reactions (Table 6.1) in a 10μl reactions containing 5μl 2x QiagenMultiplex PCR Master Mix, 200pmol of each primer and approximatly 40ng template DNA. The thermal cycling protocol was 15min at 94° followed by 30x (30s at 95°C; 45s at 58°C and 1min at 72°C) and ended with a 30min extension at 68°. All PCR products from each individual larvae/adult were pooled for fragment analysis.

6.2.5 Data analysis

Allelic diversity (Table 6.1) was calculated for all 436 samples (including adults (n = 20) and larvae (n = 416)) in GenAlEx v6 (Peakall & Smouse, 2006).

Because the genotype of the maternal colony of each larva collected in the *ex situ* fertilisation trails was known, allele combinations for the seven polymorphic loci allowed manual paternity assignment to one of the (two or four) possible parental colonies within each tank system. However, five of the seven loci revealed Mendelian expectations for genotypic ratios only when unexpected offspring genotypes were considered heterozygotes for null alleles. Thus null alleles were considered to be present if a mismatch between a homozygote maternal genotype and homozygote larva genotype was identified. Equally, null alleles were considered to be present in larvae that seemingly lacked a paternal allele at a locus, but to which fatherhood could be attributed to a specific colony according to the genotypes at other loci. Based on the assigned paternal colony of each larva, it was possible to calculate the proportion of larvae that were selfed vs. outcrossed in the in the tank experiment.

Self-fertilisation in *G. favulus* has been hypothesised to be linked to the availability of non-self sperm and adult density, whereby selfing might be higher where there are fewer con-specifics within a population (Heyward & Babcock, 1986; Miller & Mundy, 2005). A delay in sperm release of a colony should promote outcrossing of their own eggs if conspecific sperm is available, while on the

Table 6.1: Primers and annealing temperature used for polymerase chain reactions (PCR) for each of seven microsatellite loci developed in this study and used to genotype colonies and larvae of *Goniastrea favulus* from One Tree Reef. ∗null alleles present.

Locus	**Primer sequence**	**Size of sequenced allele**	**Repeat**	**Anneal. temp. (°C)**	**No. of alleles**	**Size range of alleles**	**Group**	**Colour**
Gf–10	F: CAGGTTTCCCAGACAACCAC R: TTTGGCAGTTATTTACTCAATGTG	157	ACTG	58	5	141–157∗	G3	6-FAM
Gf–14	F: GCTTTAAGTTATGATGCCGCC R: GGCACAGACATGAGGTGGTT	211	AGTC	58	14	195–247∗	G4	6-FAM
Gf–25	F: TGGCTTTGCTCGATCTTACC R: TACGTTTGTTCGTTCGCTCA	286	AACG	58	13	251–307∗	G4	6-FAM
Gf–27	F: GGGATTCTGCATTGATCTGC R: CGACTGACTCACTGACTGACG	357	AGTC	58	5	329–357	G3	6-FAM
Gf–7A	F: GACCGACTGATTTACGGACG R: TTCTTCGCTCATCTCTTCCTG	138	ACTG	58	11	110–150	G1	VIC
Gf–17	F: GCTGTAGTCTTCCACGTCCTTC R: CCGTACAATTGGTTTCTCGC	223	AATAC	58	9	178–223∗	G2	PET
Gf–26	F: TATCGATCCCAGAAGCCTTG R: AGCGAGCTCAGTTGTGGAAC	318	AAGAC	58	15	274–368∗	G2	PET

other hand earlier sperm release should increase fertilisation success but may lead to higher selfing. I compared the female outcrossing proportions and calculated Welchs t-test using the statistical package R in order to access if variations in self-fertilisation were linked to the number of colonies within tanks (as a proxy of density), or the time of egg or sperm release. In addition I compared fertilisation success of males based on the time of sperm release.

Unfortunately, the DNA of adult colonies sampled for larvae *in situ* failed to amplify successfully, most likely due to the age of the samples (collected in 2002 and stored ultra-frozen). Nevertheless, genotyping of 9-12 larvae per colony and high polymorphism of microsatellites enabled the approximation of the maternal alleles at most loci, whereby the maternal allele(s) were derived based on the allele frequencies of the larvae and larvae were expected to share one of two possible maternal alleles at each locus. Similar to the *ex situ* experiment, null alleles were incorporated to explain unexpected allele frequency departures from Mendelian expectations. However, outcrossing was only assumed to have occurred if a larva had an allele at one or more loci that could be confidently excluded to be of maternal origin considering the allele frequencies of the entire brood.

6.3

RESULTS

6.3.1 *Ex situ* self-fertilisation trials:

Of all the larvae successfully genotyped from the tank experiment (n = 234), 29.9% were self-fertilised, with an average of 28.5% (±25.4 SD) of larvae from each colony being selfed (Table 6.2). However, individual selfing proportions were very variable. Colonies in tanks with two colonies showed a higher average selfing rate (43.4%±30.1 SD, n = 5) than those in tanks with four colonies (20.3%±21.5 SD, n = 9) suggesting that selfing may be density related, however, this difference was not significant (Welch two sample t(6.332) = 1.5, p = 0.18). Equally, there was no significant difference in the selfing rate between colonies that released eggs earlier (average selfing: 34.4%±26.1 SD, n = 7) and those that released eggs later (average selfing: 22.7%±27.3 SD, n =7) (Welch two

Table 6.2: Summary of results from the *ex situ* fertilisation trial. Proportions of fertilisation shown in percent (rounded).

Tank	**Colony**	**Number of larvae genotyped**	**Time eggs released (pm)**	**Time sperm released (pm)**	**Proportion of colony's eggs outcrossed**	**Proportion of colony's eggs self-fertilised**	**Proportion of all non-natal larvae collected from tank fertilised by colony's sperm**
Tank 1	AD1	19	3:00	3:05	37%	63%	10%
	AD2	10	3:07	3:30	90%	10%	8%
	AD4	20	3:06	3:42	85%	15%	12%
	AD3	0	none	3:08	N/A	N/A	56%
Tank 2	AD5	20	3:30	4:07	75%	25%	3%
	AD6	10	3:25	3:32	100%	0%	67%
	AD7	19	3:25	3:42	100%	0%	0%
	AD8	0	none	3:42	N/A	N/A	33%
Tank 3	AD9	20	3:34	3:44	70%	30%	78%
	AD10	16	3:45	3:55	100%	0%	27%
	AD11	10	3:33	3:46	60%	40%	16%
	AD12	0	4:00	3:57	N/A	N/A	2%
Tank 4	AD13	19	3:01	3:14	68%	32%	21%
	AD14	19	3:05	3:10	21%	79%	68%
Tank 5	AD15	0	3:40	3:42	N/A	N/A	100%
	AD16	18	none	3:40	100%	0%	N/A
Tank 6	AD17	0	4:03	4:05	N/A	N/A	56%
	AD18	18	3:45	4:00	56%	44%	N/A
Tank 7	AD19	16	3:00	3:30	38%	62%	N/A
	AD20	0	3:25	4:06	N/A	N/A	38%

sample t(11.973) = 0.8, p = 0.43), or those that released sperm first (average selfing: 36%±32.5 SD) and those that released sperm last/later (average selfing: 23.1%±21.6 SD) (Welch two sample t(8.223) = 0.8, p = 0.42). Furthermore, although colonies that released sperm first had a higher fertilisation success (average proportion of larvae fathered was 58.6%±32.8 SD, n = 4 compared with 29.5%±28.5 SD, n = 13 in all other colonies), this trend was not significant (Welch two sample t(4.497) = 1.6, p = 0.18).

6.3.2 *In situ* self-fertilisation trials

For a total of 182 field fertilised larvae originating from 16 colonies were successfully genotyped. The average outcrossing rate *in situ* was determined to be 96.2% (±5.4 SD), recognising this is probably an underestimate due to the conservative scoring of maternal alleles (Table 6.3). The remaining 3.8% of larvae may have been produced either by selfing or outcrossing with individuals of similar allele frequencies to the maternal colony.Although the number of fathers for each brood could not be determined exactly, the allelic diversity for most broods suggested paternity by multiple individuals.

6.4

Discussion

Self-fertilisation may not be an important life-history strategy in the coral *Goniastrea favulus* as had been thought initially. Despite considerable experimental evidence of self-compatibility (Heyward & Babcock, 1986; Stoddart et al., 1988; Miller & Mundy, 2005), we found selfing rates on the reef to be low. Indeed the fact that the laboratory selfing rate for *G. favulus* was almost ten-fold higher than selfing under natural conditions suggests that laboratory experiments may fail to reflect real world conditions and lead to a distorted perception of reproductive relationships within and among coral species.

6.4.1 Does the ability to self-fertilise result in high fertilisation success in *G. favulus*?

Very high *in situ* fertilisation success of up to 94% has been reported for *G. favulus* on the reef flats of OTI (Miller & Mundy, 2005). Miller & Mundy (2005) speculated that this finding may be partly attributed to the self-compatibility of *G. favulus*, which has been considered a mechanism to increase fertilisation success if conspecific sperm is rare or absent (Heyward & Babcock, 1986). However, our findings indicate that self-fertilisation is rare suggesting that there is no link between self-compatibility and fertilisation success in *G. favulus* at OTI. This also supports findings of a population genetic study that showed little evidence of inbreeding in the same populations at OTI (Miller & Mundy, 2005).

The reef flat populations of *G. favulus* at OTI are characterised by high colony densities (>1.5 colonies/m^2; Miller & Mundy, 2005). Although sperm is considered limiting in free spawning organisms (Levitan & Petersen, 1995) it may be that sperm concentrations within high density populations of *G. favulus* at OTI facilitate outcrossing, hence the limited evidence of selfing and inbreeding found in these populations (Table 6, Miller & Ayre, 2008a). For the bryozoan *Celleporella hyalina* selfing rates were negatively correlated with the abundance of outcross sperm and possible mating partners (Yund & McCartney, 1994), and the lower average selfing rates in tanks with four colonies versus those with two colonies indicate that same may be true for *G. favulus*, however, further experiments are needed to verify if the selfing rate is indeed negatively related to the number of possible mating partners.

Table 6.3: Summary of results from the *in situ* fertilisation trial.

Colony	**Number of larvae genotyped**	**Minimal proportion of outcrossing**	**Maximum proportion of selfing**
AD2.26	11	100%	0%
AD2.52	12	100%	0%
AD2.68	12	100%	0%
AD2.70	10	100%	0%
AD1.3	12	100%	0%
AD1.40	10	100%	0%
AD1.47	12	100%	0%
AD1.4	11	82%	18%
AD2.66	11	91%	9%
AD2.29	11	100%	0%
AD2.31	12	92%	8%
AD2.4	11	91%	9%
AD1.33	12	92%	8%
AD1.68	12	92%	8%
AD2.71	12	100%	0%
AD1.114	11	100%	0%

6.4.2 Does spawning behaviour influence self-fertilisation rates in *G. favulus*?

In the tank experiments, eggs were released by colonies either synchronously or within 30min of each other (Table 6.2), with sperm being released subsequently. The final maturation division of the oocytes and the release of polar bodies occurs 15 to 30min after egg release (Heyward & Babcock, 1986). Consequently, by the time eggs were able to be fertilised most colonies in a tank had released sperm which had dispersed within the tanks due to their relatively small volume and thus eggs were exposed to self- and non-self sperm simultaneously. While there was some evidence that early sperm release resulted in higher fertilisation success within our experimental chambers (Table 6.2), there was no indication from our data that the mode of spawning in *G. favulus* whereby eggs are generally released before sperm (Quinn & Kojis, 1981; Miller & Mundy, 2005) helps to prevent self-fertilisation. Certainly when confronted with a choice between self and non-self sperm in the laboratory, *G. favulus* colonies competed successfully with conspecifics for the fertilisation of their own eggs.

G. favulus at OTI occurs on the reef flats in habitats that pond during low tide, thus forming small, isolated pools during the time when the corals are spawning and thus maintaining coral tanks during the annual spawning for the *ex situ* experiment simulated similar conditions as in the wild. However, water flows driven by wind or tidal water flow may lead to a higher mixing and dispersal of sperm promoting less selfing. At this point it remains unclear what lead to the high discrepancy between selfing rates *in situ* and *ex situ*. Future experiments aiming to understand sperm mixing and dispersal distances in natural populations may help to provide a better understanding of mechanisms that reduce self-fertilisation on the reef.

6.4.3 Evolutionary significance of self compatibility in *G. favulus*

Generally self-compatibility seems to be rare in corals and other marine species (Carlon, 1999), which follows the widely held theory that outcrossing should be favoured and mechanisms should be in place to avoid selfing and the negative effects of inbreeding depression (Williams, 1975). For example, in the solitary ascidian *Ciona savignyi* non-self sperm out-competes self sperm due to components in the chorion (Jiang & Smith, 2005) and in many coral species there are barriers that prevent self sperm from fertilising eggs (e.g. Miller & Babcock, 1997; Willis et al., 1997). Yet, the ability of *G. favulus* to readily self-fertilise (Stoddart et al., 1988) and particularly my results that show high selfing rates *ex situ* even in the presence of non-self sperm (Table 6.2) indicates no such mechanism exists in *G. favulus* and prompts questions of the evolutionary significance this life history strategy plays in this species. Despite earlier suggestions that selfing may increase fertilisation success and thus the fitness of populations, this study has shown little importance of selfing for fertilisation in natural high-density populations, Nonetheless, the ability to self-fertilise is clearly maintained in *G. favulus*, suggesting the ability to self-fertilise may be important in some situations, for example in low density populations where mating partners are few, or absent, and indeed there was some indication in our data that selfing rates were higher when colony densities in tanks were low.

The persistence of selfing in *G. favulus*, even when it appears to contribute little to the maintenance of populations is an evolutionary anomaly not unlike that seen in the asexually brooding coral *Pocillopora damicornis* whereby considerable resources are put into generating asexual larvae but clonal recruits are rarely found (Ayre & Miller, 2004; Sherman et al., 2006). Both asexual reproduction and self-fertilisation may be strategies to facilitate reproduction if mating partner are limited or absent. Consequently, to better understand why such unusual reproductive strategies persist in corals, it will be important to investigate reproduction, recruitment and population structure in situations where mating partners are limited or absent.

6.4.4 Inference of reproductive relationships using laboratory trials

The ten-fold difference in self fertilisation *ex situ* compared with *in situ* suggests that the importance of selfing in *G. favulus* may have been considerably overestimated based on laboratory trials. It is possible, therefore, that predictions of other reproductive patterns, processes and relationships within and between coral species based on similar laboratory fertilisation trails should be considered with caution. For example, although laboratory trials may reveal if fertilisation compatibility exists i.e. between coral species, these trials may fail to reflect the frequency of such reproductive outcomes in natural situations. This finding may not be limited to self-compatibility, but likely extends to inter-specific gamete compatibilities. Cross-fertilisation compatibility has been described for several marine species, including corals (e.g. Miller & Babcock, 1997; Willis et al., 1997), sea stars (e.g. Harper & Hart, 2005) and molluscs including abalone (e.g. Brown, 1995; Lafarga de la Cruz & Gallardo-Escárate, 2011) and laboratory fertilisation trials in corals suggest that inter-specific hybridisation maybe common in corals (Willis et al., 1997). While hybridisation among some coral species certainly occurs (Willis et al., 2006, , Chap. 5) and represents an important evolutionary process (see Willis et al., 2006), laboratory trials may have lead to an over estimation of its frequency and significance at ecological time scales. As shown here, observing gamete compatibility under laboratory conditions may not necessarily reflect its occurrence in the real world.

CHAPTER
SEVEN

DISCUSSION

Investigating the evolutionary and ecological roles of different reproductive cycles and their impact on dispersal and population structure, this thesis further emphasises the complexity and diversity of life history strategies in corals.

At the population level, the data presented here suggests that the role and importance of life history strategies such as asexual brooding or self-fertilisation may have been misinterpreted or overestimated in the past. For example, for *P. damicornis* previous studies have found little evidence for local clonal population structures in Eastern Australia (Benzie et al., 1995; Miller & Ayre, 2004; Sherman et al., 2006), although asexual brooding seems to be common (Ayre & Miller, 2004, Chap. 2, 3). Indeed, the data presented here strongly suggests that the hypothesis, ***P. damicornis* (or hidden species within) brood larvae in Eastern Australia which are solely asexual**, can be confirmed. Contradicting conventional evolutionary models (e.g. Williams, 1975), the difference in characteristics of spawned and clonal brooded larvae revealed here, suggests that the latter are predominantly used for long distance dispersal and serve to spread the genotype (Chap. 3). Similarly, observations of high self-compatibility for *Goniastrea favulus* in laboratory trials (Stoddart et al., 1988) have lead to an overestimate of the importance of this strategy in natural populations (Chap. 6). Indeed, my findings challange the hypothesis that **self-fertilisation represents an important reproductive strategy in *Goniastrea favulus*** at least for natural populations. However, confirming previous laboratory trials (Stoddart et al., 1988), the laboratory experiments conducted here don't support the hypothesis that ***G. favulus* favours non-self over self-fertilisation in situations of sperm choice**. From an evolutionary perspective, the persistence of these rarely used reproductive strategies may increase reproductive flexibility, by allowing genotypes to reproduce in the absence of conspecifics and to escape isolation. However, these examples stress the difficulty of predicting population structures based on life history strategies.

At the species level, the previously reported reproductive plasticity within *P. damicornis* at different geographic locations seems to be linked to the cross-sampling of different hidden species that harbour different reproductive modes (Chap. 2), and although this reproductive plasticity may have initially driven speciation (Chap. 3), genetic data indicates that occasional introgressive hybridisation remains possible even among morphologically and reproductively divergent species (Chap. 2–5). However, the hypotheses that ***P. damicornis* consists of a species complex rather than a single reproductively and morphologically plastic species** and that **the genetic lineages identified within *P. damicornis* correspond to taxonomical morphospecies** can be confirmed. However, hybridisation or reticulation in corals seems to occur at evolutionary rather than ecological time scales, with natural populations possibly being far less exposed to hybridisation then previously assumed (Chap. 6). The lack of strict species boundaries complicates coral systematics and taxonomy. Yet, hybridisation seems to increase the evolutionary plasticity of species and may increase the potential for adaptation (Seehausen, 2004).

Overall, this thesis brings us one step further in understanding the processes of speciation, underlying the evolution of species within corals, using the genus *Pocillopora*. Independent evolutionary units confined by permeable species boundaries forming syngameons, may be common in corals making them ideal organisms to study these fundamental evolutionary processes.

7.1

FROM POPULATION TO SPECIES LEVEL: REVISITING POPULATION STRUCTURES IN CORALS OF THE GENUS *Pocillopora*

In the past three decades, many studies have investigated population structures of *Pocillopora damicornis* (Stoddart, 1984a; Stoddart, 1984b; Ayre & Hughes, 2000; Ayre & Miller, 2004; Miller & Ayre, 2004; Sherman et al., 2006; Whitaker, 2006; Miller & Ayre, 2008b; Souter, 2010; Yeoh & Dai, 2010; Torda, 2013) and other *Pocillopora* species (e.g. Ridgway et al., 2001; Magalon et al., 2005; Ridgway et al., 2008; Pinzón et al., 2012). Although extensively studied, many basic biological and ecological aspects of species such as *Pocillopora damicornis* have remained confusing. For example, primarily sexually derived population structures are a mismatch to the frequent asexual brooding observed for this species in Eastern Australia (Ayre & Miller, 2004). In addition, the origin of sexual larvae remained controversial. My discovery of five hidden species that are reproductively divergent within what has previously been considered a single species, *P. damicornis*, may well explain some of these discrepancies (Chap. 2). Correct species identification is vital, especially as microsatellites markers identified for *Pocillopora* species seem to amplify most taxa in the genus (e.g. Pinzón et al., 2013) and thus the potential for mixed species sampling and continued confusion about population structure and processes remains high without proper taxonomy.

7.1.1 Reproductive mode and local population structures

7.1.1.1 Is brooding in *Pocillopora* exclusively asexual?

Here I provide further support for the clonal generation of larval broods of brooding *Pocillopora* species in Australia, confirming previous studies using allozymes (Stoddart, 1983; Ayre & Miller, 2004; Sherman et al., 2006). However, *P.* cf. *damicornis* in Taiwan has been reported to brood partially sexual broods (Yeoh & Dai, 2010). Considering the observations presented here, it may be more likely that the inconspicuous sexual spawning (Chap. 3) may simply have been missed in Taiwan. The few sexual larvae reported within largely clonal broods may reflect 1.) contamination of clonal broods with sexual spawned larvae, 2.) somatic mutations (e.g. Oppen et al., 2000), or 3.) chimerism (e.g. Hidaka, 1985).

Contamination of asexual broods with sexual larvae produced from broadcast spawning is a possibility, especially when collecting *in situ* with nets as it was the case in Yeoh & Dai (2010). However, the clear size difference between sexual planulae (based on the size of the eggs) and brooded planulae should make it easy to distinguish between the two different types in future experiments (Chap. 3).

Somatic mutations have recently been suggested to present a major source of genetic heterogeneity within a single colony (Puill-Stephan

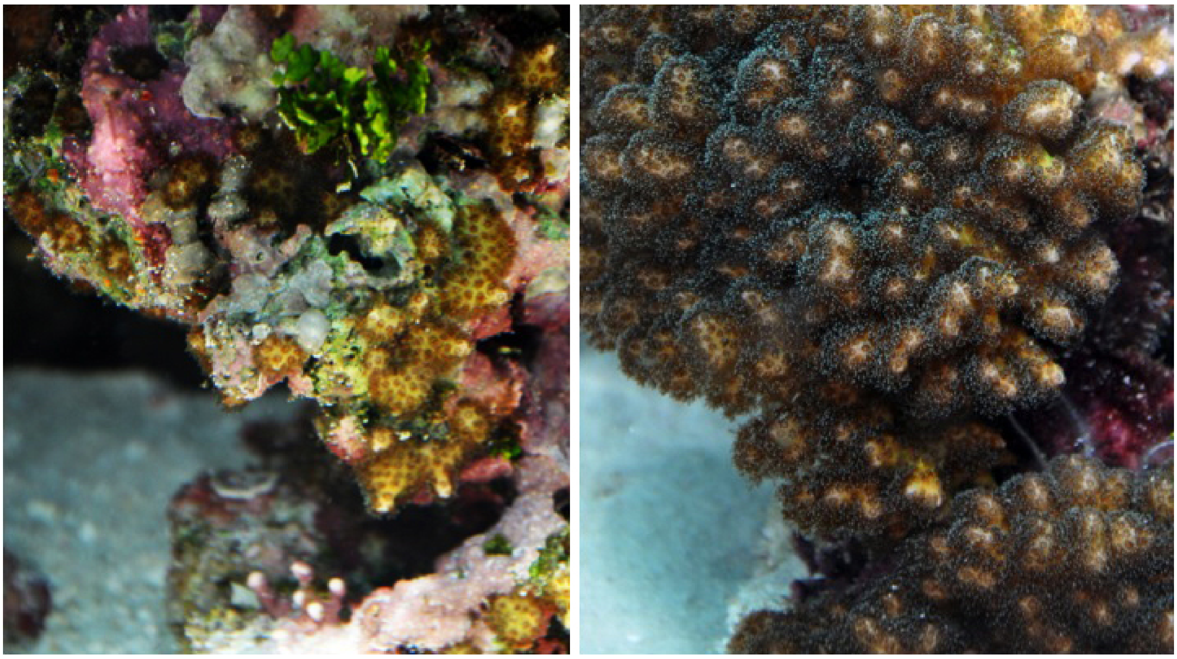

Figure 7.1: Photos of partially fused pocilloporid recruits. Left: About 1-3 months after settlement. Right: 8 months after previous photo was taken.

et al., 2009; Oppen et al., 2011; Maier et al., 2012; Puill-Stephan et al., 2012), with as many as 17% of colonies found to be genetically heterogeneous (Maier et al., 2012) which may be a source of post settlement adaptation (Oppen et al., 2011). Consequently, somatic mutations could have the potential to encrypt clonal reproduction by generating multi-genotype colonies or novel larval genotypes in the absence of sexual reproduction, although as yet this has not been observed in the brooded larvae of *Pocillopora* spp. on the GBR.

In addition, to genetic heterogeneity associated with mutations, colonies may harbour different genotypes due to chimerism. For example, in *Stylophora pistillata* planulae are known to aggregate when settling (70% of planulae settled in aggregations (Amar et al., 2007)) and multiple genotype aggregations have higher rates of survival (Amar et al., 2008). *P.* cf. *damicornis* larvae originating of the same colony fuse without rejection (Hidaka et al., 1997) and *P. acuta* recruits follow similar patterns (personal observation; Fig. 7.1, although the origin of larvae (sexual or asexual) in this instance is unknown). Considering brooded larvae of *P. damicornis* and *P. acuta* seem to be exclusively clonal, aggregated settlement is not surprising as allogeneic responses can be excluded. However, the aggregation of siblings generated by sexual reproduction may lead to mosaicism, and ultimately the perception of genetic diversity in larval broods. Although aggregated settlement may increase survival rates, settling next to relatives should also increase inbreeding and inbreeding depression (Grosberg, 1987). Consequently these strategies may explain the heterozygosity deficits observed in *P. damicornis* in population genetic studies (e.g. Miller & Ayre, 2004; Sherman et al., 2006).

7.1.1.2 The roles of asexual and sexual progeny

Confirming previous hypotheses of a mixed mode of reproduction in *Pocillopora damicornis* inferred from histology (Muir, 1984; Ward, 1992), here I present the first spawning observation for this species illuminating the source of sexual larvae in brooding *Pocillopora* species. Clear size and behavioural differences between brooded larvae and larvae produced from broadcast spawning suggest different roles of each larval type within the life cycle of these species.

Clonal brooding, in addition to the ancestral broadcast spawning, seems to be apomorphic to a clade within *Pocillopora* (comprising *P. damicornis*, *P. acuta* and *P. aliciae*; Chap. 3, 6). However, populations of brooding *Pocillopora* in Eastern Australia are predominantly sexually derived (Benzie et al., 1995; Ayre & Hughes, 2000; Ayre & Miller, 2004; Sherman et al., 2006), which raises the question of the role asexual brooding plays in these complex life histories (see Ayre & Miller, 2004). In contrast to Eastern Australia, populations of brooding *Pocillopora* species in Taiwan and Western Australia show high clonal structures with only a few genotypes dominating entire habitats (Stoddart, 1984a; Whitaker, 2006; Yeoh & Dai, 2010). Although these population structures fit with asexual brooding, the clonal structures could have been equally generated by asexual fragmentation as shown in a recent study. Pinzón et al. (2012) identified high clonal structures in sheltered environments, contrasting high genotype diversities at exposed sites for a non-brooding *Pocillopora* species, suggesting that the impact of asexual reproduction may be strongly influenced by environmental conditions. However, the only method to identify if asexual genotypes are derived from fragmentation or asexual brooding is to investigate larval recruitment patterns.

Using species-specific markers and microsatellites jointly developed with Torda and co-authors 2013, Torda (2013) identified for the first time species-level recruitment rates for two pocilloporids (*P. damicornis* and *P. acuta*). This major achievement revealed that the percentage of clonal recruits within reefs in Eastern Australia reflects approximately the clonality level in the adult populations for both species (percentage of clonal recruits on recruitment tiles was 15% for *P. damicornis* and 7% for *P. acuta*) (Torda, 2013). Torda (2013) could also show that recruitment was mostly philopatric with 48%-84% local recruitment of larvae for *P. damicornis* and 39% local recruitment for *P. acuta* supporting previous studies of population genetic structure (Benzie et al., 1995; Ayre & Hughes, 2000; Ayre & Miller, 2004; Sherman et al., 2006). These findings also confirm patterns observed for *P. acuta* by Souter (2010) in African waters. Consequently, at least in Eastern Australia, asexual broods seem to contribute comparably little to local population structure and the dilemma remains as to why these species invest such a large amount of energy into a reproductive mode that does not appear to contribute to recruitment (Ayre & Miller, 2004).

Torda (2013) found clone pairs at various scales with the furthest being 444 km apart, his findings further support my hypothesis that sexual progeny from broadcast spawning settle predominantly locally and the larger, and better-provisioned asexual larvae are more widely dispersed (Chap. 3). Alternatively, the observed proportions of clones and sexually derived individuals may reflect the fecundity associated to each reproductive strategy, i.e. the quantity of progeny derived from spawning may simply dominate the progeny derived from asexual brooding. From an evolutionary perspective, asexual brooding allows for reproduction in the absence of conspecifics and, probably more importantly, the return into the planktonic phase. Consequently, via asexual brooding an individual can escape isolation and disperse towards potential mating partners. Asexual brooding may thus increase the fitness of a genotype by increasing the number of possible mating partners.

7.1.2 Allopatric specification in *Pocillopora* species

7.1.2.1 Connectivity in brooding *Pocillopora* species

The hypothesised contrasting roles of sexual and asexual progeny (Chap. 3) within the life cycle of brooding *Pocillopora* species agree well with previous observations of connectivity at larger geographical scales. For example, for the asexual brooders *P. damicornis* and *P. acuta* microsatellite data (Torda, 2013) confirmed the high genetic similarities over large spatial scales (presumably provided by far dispersing asexual larvae) and strong genetic differentiation at local scales in the GBR (presumably provided by local sexual recruitment) observed previously using allozyme data (Ayre & Hughes, 2000; Ayre & Miller, 2004; Sherman et al., 2006). However in contrast to high levels of gene flow along the Great Barrier Reef (Benzie et al., 1995; Ayre et al., 1997; Ayre & Hughes, 2000; Torda, 2013), the population structure of *P. damicornis* in subtropical Eastern Australia seems to be more complex. As presented in Noreen et al. (2013), we found high genetic structures in the peripheral populations of subtropical Eastern Australia based on microsatellites and DNA sequence data supporting earlier findings using allozymes (Miller & Ayre, 2008b). Besides the seemingly endemic species *P. aliciae* in the Solitary Islands described in Chap. 4, the population of *P. damicornis* at Lord Howe Island was also significantly differentiated from the GBR *P. damicornis* (Fig. 7.2) based on mitochondrial regions. However, the level of genetic differentiation is too low to consider the Lord Howe Island population as a separate species (Chap 2) but may reflect ongoing allopatric speciation. However, the genetic divergence of the peripheral populations in the Solitary Islands and at Lord Howe Island supports the idea that evolutionary novelty may be found at the edge of a species distribution (Budd & Pandolfi, 2010).

Interestingly, whereas the symbiont signature was identical for different *P. damicornis* specimens sampled along the GBR (Noreen et al., 2013, , Chap. 2), subtropical individuals were associated with endemic symbiont types (Chap. 2, Wicks et al. 2010; Noreen et al. 2013) (Fig. 7.2). Pocilloporids transmit their symbionts maternally leading to apparent species-specific associations (Pinzón & LaJeunesse, 2010). However, recent research conducted on Eastern Pacific *Pocillopora* indicates that associations may be more flexible then previously assumed (Cunning et al., 2013) and the mode of transmission may not necessarily limit the symbiont diversity (Oppen, 2004; Byler et al., 2013). Considering the striking constancy of symbionts associated with *P. damicornis* along the GBR, divergences in symbiont signature may be indicative of adaptive processes at evolutionary time scales rather then individual-based ecological time scale responses (see Cunning et al., 2013). Supporting this hypothesis, even in the subtropical locations of Eastern Australia the diversity of different symbiont signatures within species was limited (Wicks et al., 2010; Noreen et al., 2013).

From a population perspective, symbiont association may play a key role in limiting latitudinal dispersal of *Pocillopora* larvae and driving the observed levels of isolation. Indeed, the isolation of the subtropical populations is surprising, considering the strong southward-directed Eastern Australian Current (Fig. 7.2) and larvae competencies of several weeks (see Chap. 1). Both should support high levels of gene flow from the Great Barrier Reef. If, however, flexibility of symbiont association is restricted, migrants may suffer from less beneficial GBR-symbionts at the southern locations and consequently have lower fitness (Noreen et al., 2013).

7.1.2.2 Connectivity in non-brooding *Pocillopora* species

Considering the similarity of sexual larvae between brooding and non-brooding *Pocillopora* species (Chap. 3), both should have similar dispersal characteristics with predominant local dispersal. Indeed, high local population structures and philopatric recruitment is described for a non-brooding *Pocillopora* species in the Tropical Eastern Pacific (e.g. Combosch & Vollmer, 2011). However, high gene flow over vast geographical areas has been reported for non-brooding *Pocil-*

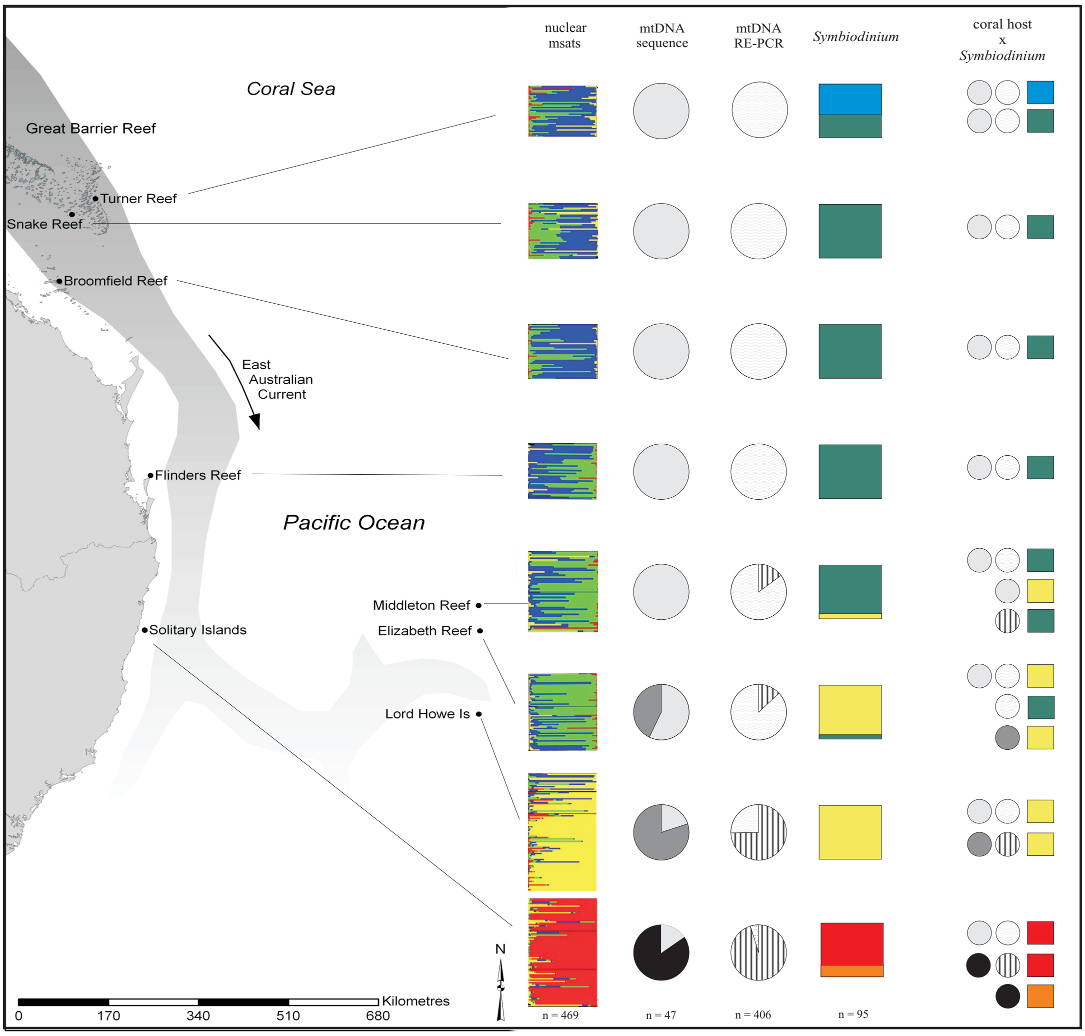

Figure 7.2: Genetic divergence among subtropical *Pocillopora* populations in Eastern Australia from Noreen et al. (2013). From left to right: Map of coastal Eastern Australia with indicated flow of the Eastern Australian Current; Microsatellite STRUCTURE plots (k= 4); identfied mitochondrial (ORF) halpotypes per population; identfied mitochondrial (ORF) halpotypes per population; identified lineages using a rapid identification assay (Torda et al., 2013); *Symbiodinium* signature based on ITS2; Summary of host/symbiont associations; circles represent host lineages (with those identified sequencing the ORF region on the left row, and those identified using the rapic identification assay on the right row), coloured quadrats indicate the respective symbiont association.

lopora species (Magalon et al., 2005; Pinzón & LaJeunesse, 2010; Pinzón et al., 2013) suggesting a high dispersal ability of spawned progeny within these species. Indeed, the non-brooding *P. verrucosa* seems to be the geographically most widely distributed species within the genus (Pinzón et al., 2013). Further, (Pinzón et al., 2013) found little variation in allelic compositions and homogenous populations of brooding and non-brooding populations reaching over thousands of kilometres, suggesting successful long-distance dispersal. Although it may need only very few migrants to maintain connectivity within a metapopulation (Hellberg, 1995), the lack of structure and an apparent homogeneous population structure identified for *P. meandrina* (Type 1), reaching from the Indian Ocean to the reefs of Panama, may rather be attributed due to a lack of intra-specific polymorphism of the seven used microsatellite markers. I believe that more research integrating further microsatellite markers or SNP technology is needed to test this finding. In addition, research on the larval biologies of these different species is needed to truly understand the differences of their population genetic structures.

7.1.3 Inter-specific gene flow in *Pocillopora* species

In addition to genetic divergence and evolutionary novelty, isolation (such as found for populations in remote island systems) has also been proposed to promote interspecific hybridisation in corals (Richards et al., 2008). Richards et al. (2008) suggested that some rare *Acropora* species in isolated island systems may rather be hybrids. Furthermore, Miller & Ayre (2004) identified a putative intergeneric hybrid between *P. damicornis* and *Stylophora pistillata* at Lord Howe Island. However, as stressed throughout this thesis, at the metapopulation lineage level, species within *Pocillopora* have independent evolutionary histories and directions. Comparing different *Pocillopora* lineages using microsatellites, Pinzón et al. (2013) found well defined population structures further supporting the species boundaries identified here (Chap 5). In addition, Pinzón et al. (2013) found little support for recent hybridisation events between species. However, limited nuclear divergences indicate that inter-specific hybridisation events occasionally occur (Chap. 2). For example, *P. damicornis* and *P. verrucosa* lack divergences in the nuclear ITS2 region, although these species are genetically divergent in the mitochondrial genome (Chap. 2), maintain different reproductive traits (Chap. 3) and show strict divergences at the corallite structure (Chap. 4, 6). The mitochondrial sequences suggest that *P. acuta* is much more closely related to *P. damicornis* than *P. damicornis* to *P. verrucosa*. But *P. acuta* is strictly divergent to both species in the nuclear regions (ITS2; HSP70) in Australia. This strongly suggests that a lack of nuclear divergences may rather be attributed to introgressive hybridisation between *P. damicornis* and *P. verrucosa*, than to incomplete lineage sorting. Hybridisation among coral species is most likely important at evolutionary rather then ecological time scales and may be generally limited to introgressive hybridisation.

Observing geographically restricted morphological characteristics within single coral species, Veron (1995) described coral races as populations within a metapopulation lineage depicted by varying levels of isolation and local adaptation. These create what he called “reticulate networks”, considering that gene flow exists at evolutionary time scales. Interestingly, although clearly differentiated in Australia *P. damicornis* and *P. acuta* lack clear nuclear divergence in Hawaii (Flot et al., 2008) while still maintaining opposite lunar reproductive cycles (Richmond & Jokiel, 1984). Consequently, it seems that introgressive hybridisation events and thus secondary contact between species can be restricted to certain geographical locations/regions, without necessarily impacting the entire metapopulation lineage. This locally restricted exchange of genes in the form of introgression may further contribute to regionally unique morphological characters and ultimately explain intra-specific morphological divergences observed between individuals from distant locations. Thus, in addition to local adaptation, introgressive inter-specific hybridisation may contribute to the evolution of races within corals (Fig. 7.3) and may confuse species recognition in different geographical regions.

In contrast to nine species described for the Tropical Eastern Pacific (TEP) (*P. damicornis*, *P. verrucosa*, *P. meandrina*, *P. eydouxi*, *P. inflata*, *P. captitata*, *P. effusus*, *P. elegans*, *P. ligulata*; Veron (2000)), only three clearly divergent genetic lineages were identified, of which one seems to be endemic to Clipperton and Galapagos (Flot et al., 2008; Pinzón & LaJeunesse, 2010). If the TEP is really restricted to only three species as suggested (Flot et al., 2008; Pinzón & LaJeunesse, 2010), it

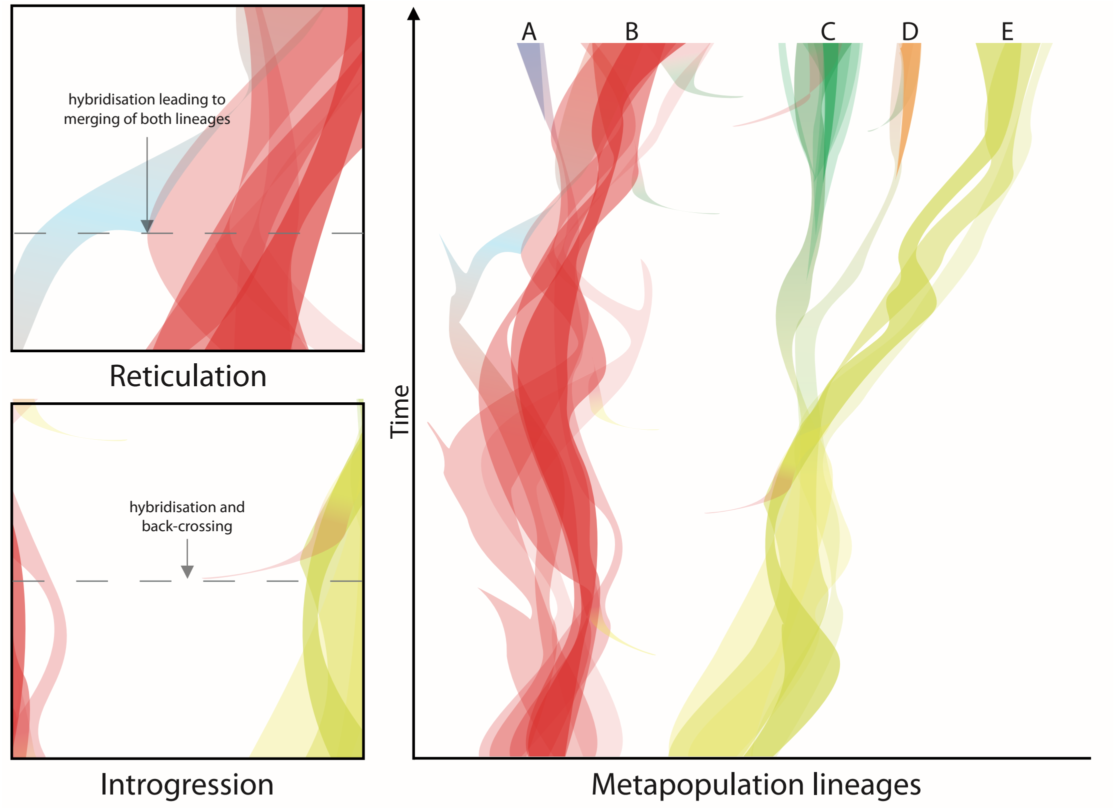

Figure 7.3: Right: Schematic illustration of divergences of metapopulation lineages over time, undergoing processes of introgression and reticulation. Left: Schematic illustration of reticulation and introgression.

is striking that gross morphologies associated with distinct species in locations with high species diversity such as the GBR (Chap. 5) or Hawaii (Flot et al., 2008) are seemingly exhibited by few very plastic species in low diversity systems (Pinzón & LaJeunesse, 2010). Besides the endemic species (identified as *P. effusus* by Pinzón et al., 2013), genetic data indicates that *Pocillopora* Type 1 (Pinzón et al., 2013) and *P. verrucosa* (Type 3 Pinzón et al., 2013) are found in the TEP (Fig. 5.5). *P. verrucosa* seems to be very plastic throughout its global distribution and it is not surprising that it has been identified as *P. damicornis* in the TEP given its *damicornis*-like morphology in other regions including Australia (see Chap. 5). In sharp contrast, *P. meandrina* is very well defined in Australia (Chap. 5), while in the TEP it seems to show a much higher phenotypic plasticity with apparent *damicornis*-like morphologies. Following the ecomorph-concept, phenotypes are altered to adapt to specific habitats. Consequently, high species diversity and competition may result in narrower niches limiting the phenotypic plasticity exhibited by either one species, whereas low species competition situations may result in high phenotypic plasticity. Thus, the "niche-variation model" (Valen, 1965) may apply for these coral species. Indeed, due to the lack of morphological features with strict diagnostic value, few morphological changes are needed to create morphs that appear to fit different species. As shown in this thesis, *P. damicornis* was used incorrectly as a label for various species because "a lack of verrucae" was commonly used as its defining character.

Pocilloporid species in Australia can be associated with certain habitats/niches, while some occur in sympatry with well-defined differences

(Fig. 7.4, Chap. 5). Thus, although adaptive processes following the "niche-variation model" (Valen, 1965) may be fundamental drivers underlying the apparent intra-specific morphological plasticity of species in isolated/low species diversity locations, some of the local variability may rather be attributed to introgression events in the past. *Pocillopora* Type 1 described by Pinzón & LaJeunesse (2010) in the Tropical Eastern Pacific (which appears to be genetically *P. meandrina*) exhibits morphs that seemingly fit five species (*P. damicornis, P. verrucosa, P. capitata, P. meandrina, P. eydouxi*) (Pinzón & LaJeunesse, 2010). Although Pinzón & LaJeunesse (2010) found their Type 1 and Type 3 to be well distinguished in their population structure and nuclear regions, introgressive hybridisation with migrants from Central Pacific species/populations in the past may have influenced the exhibited phenotypic plasticity within, and similarity between, species in the TEP. New genomic approaches may elucidate if genes responsible for gross morphology are shared between different *Pocillopora* species. In addition, further investigations on fine scale morphology (Chap. 5) may help to elucidate the evolutionary history of the TEP species.

In conclusion, species within *Pocillopora* may be considered a syngameon, a group of species that is characterised by occasional hybridisation at evolutionary time scales, while being reproductively isolated in the present (Lotsy & Cockayne, 1925). Recent research suggests that syngameons may be common in corals (e.g. in the genus *Acropora*: Oppen et al. 2002; Ladner & Palumbi 2012 and in some *Faviids*: Miller 1994b). Introgressive hybridisation events along the evolutionary histories of *Pocillopora* species within the syngameon may also explain why these species are often found as mosaic colonies without evidence of allogenic rejection. Immune system genes exchanged during hybridisation events may generate low levels of postzygotic incompatibility and ultimately conserve the permeability of species boundaries within the genus. Future research is needed to identify the role these mechanisms may have on levels of hybridisation and reticulation. However from a taxonomic perspective we may have to accept that species within corals may only present "the most clearly identifiable discontinuities in continuous variation from the population to the genus as appropriately stated by Veron (1995).

Figure 7.4: Mosaic colony of *P. acuta* and *P. eydouxi* (Photo: Michelle Jonker, AIMS)

7.2

FUTURE PERSPECTIVES: EXCITING TIMES FOR NEW GENOMIC APPROACHES

This thesis brings us a step closer in revealing the ecological and evolutionary significance of reproductive traits in coral species and in understanding their evolutionary history. Still, many unanswered questions remain and provide interesting directions for future research. For example, now that we can reliably predict broadcast spawning in species of *Pocillopora*, it will be possible to test for inter-specific hybridisation in this genus as it has been done for many other coral taxa (e.g. Miller & Babcock, 1997; Willis et al., 1997). However, as suggested in Chap. 6, laboratory experiments may not reflect natural processes, and in addition the long generation times for many coral species (i.e. *P.* cf. *damicornis* does not reach sexual maturity until at least 2–3 years of age (Birkeland, 1997)) complicate back-cross experiments with progeny that might further reveal the role of introgression. Thus, molecular biology may provide a better approach to investigate further levels of hybridisation in these corals, especially with the available new options in genomic research.

For butterflies, next generation sequencing techniques have revealed that introgression may allow for the exchange of adaptive genes between even more distant species (Dasmahapatra et al., 2012; Pardo-Diaz et al., 2012). Within the neotropical butterfly genus *Heliconius* wing colours and shapes have seemingly been exchanged via introgression. These studies illustrate the complexity of evolutionary processes that we are now in a position to explore for corals. Indeed, I predict that investigating genes determining gross morphological patterns within *Pocillopora* may lead to a similar outcome. In addition, investigating patterns of introgression using genomic techniques may further elucidate if hybridisation/introgressive events can be regionally restricted leading, as hypothesised, to coral races with phenotypic divergences within a species. Populations within a species that are characterised by introgressive events in the past have been suggested for several animal groups (e.g. Richards et al., 2008; Staubach et al., 2012). Indeed, even in human evolution, genomic research indicates that some *Homo sapiens* populations have hybridised with individuals of *Homo neanderthalensis* (Green et al., 2010), consequently forming a syngameon in the past (Holliday, 2006). Overall these processes are still poorly understood but apparent syngameons such as identified for the genus *Pocillopora* may be ideal to further investigate how common these processes may be and ultimately to elucidate their significance for evolutionary processes.

Genomic comparisons between clades within *Pocillopora* may further reveal genes linked to asexual brooding in species of clade 1 (Chap. 3). Comparisons to *Stylophora* and *Seriatopora* species may in addition reveal how asexual brooding and sexual spawning in *Pocillopora* compares to the sexual brooding within its sister genera on a genomic level. This may help to understand the evolution of these contrasting reproductive traits in the family of the Pocilloporidae and assist in further exploring the significance these traits have on the levels of reproductive isolation.

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GLOSSARY

A

Allopatric specification From allo-, meaning ”other” and -patric, meaning ”place”. Speciation that occurs when to populations of the same species became geographically isolated from each other, preventing further genetic exchange.

Allozyme From allo-, meaning ”other” and (en)zyme. A number of different structural forms of the same enzyme coded by a different allele.

Apomorphic From apo-, meaning ”away from” and -morphic, meaning ”shape”. A new evolutionary trait or character which distinguishes an organism or taxon from others that share the same ancestor.

C

Calyx (plural calices) Concave depression that houses polyp.

Cespitose Many stems from one rootstock.

Coenosteum Skeletal area between two adjacent corallites.

Corallite Skeleton produced by a single polyp.

Corallum The entire skeleton of a coral.

G

Gonochorism Having just one sex in any one individual organism.

H

Hermaphroditism Having female and male sex organs displayed in an individual organism.

Hexamerous From hexa-, meaning ”six”. A structure (here speta) symmetrically arranged in six groups.

Hybridisation Interbreeding between two different species.

I

Introgression Genetic exchange and gene flow from one species into the gene pool of another by the repeated backcrossing of an interspecific hybrid with one of its parent species.

Incomplete lineage sorting Shared ancestral genetic diversity between to different descendant species due to incomplete sorting.

P

Philopatric From philo-, meaning ”to love” -patric, meaning ”place”. The behaviour of an organism to remain near its place of birth.

Postzygotic incompatibility Reproductive isolation due to post-fertilisation hybrid incompatibility.

S

Septum (plural septa) Skeletal plates that radiate into the calyx from the wall.

Spinula (plural spinulae) Small little spines or thorns.

Subterete Almost cylindrical.

Styloid Skeletal feature that is arranged forming a stylus.

Sympatric speciation From sym-, meaning ”same” and -patric, meaning ”place”. Speciation that occurs without geographic isolation within the same geographic region.

Synapomorphic From syn-, meaning ”same, shared” and apo-, meaning ”away from” and -morphic, meaning ”shape”. A new evolutionary trait or character which distinguishes two or more taxa and orginiates from a evolutionary event in a common ancestor.

Syngameon Populations of a single species, or cluster of different species which are capable of exchanging genes directly or indirectly.

Synonymization The process of discarding a younger species name for an older, valid species name.

APPENDIX

S1

PERMITS

All necessary permits were obtained for the described field studies. Samples were taken under Permit No G10/33440.1 issued to the Australian Institute for Marine Science and Permit No G09/32395.1 issued to S. Schmidt-Roach and P. Souter by the Great Barrier Reef Marine Park Authority (GRBMPA).

Permit No 2011/158501 issued to Sebastian Schmidt-Roach by the Rottnest Island Authority.

Permit No P11/0046-1.0 issued to Sebastian Schmidt-Roach by NSW Department for Primary Industries.

MATERIALS CHAPTER 2

S2

Figure S2.1: Photos of colonies representing examples of each morphological group. Colony numbers of tropical individuals correspond to individuals sequenced.

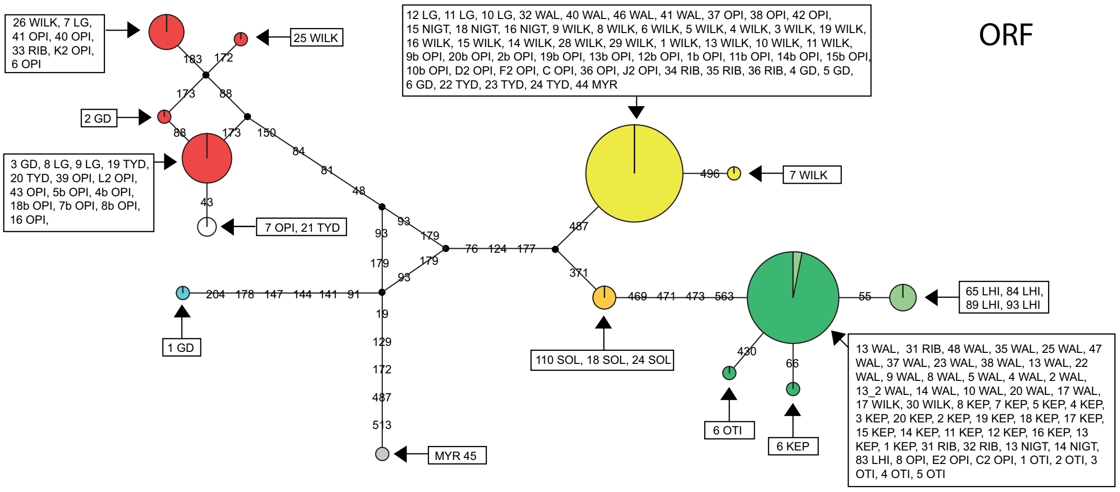


Figure S2.2: Haplotype network based on ORF DNA sequence data (n = 145, total alignment length = 592 bp)

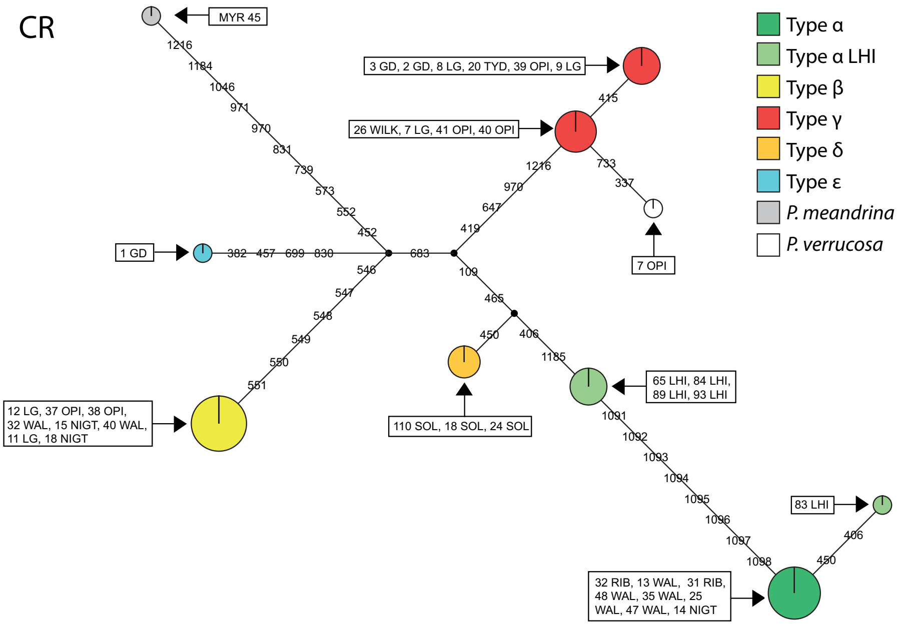


Figure S2.3: Haplotype network based on CR DNA sequence data (n = 37, total alignment length = 1266 bp).

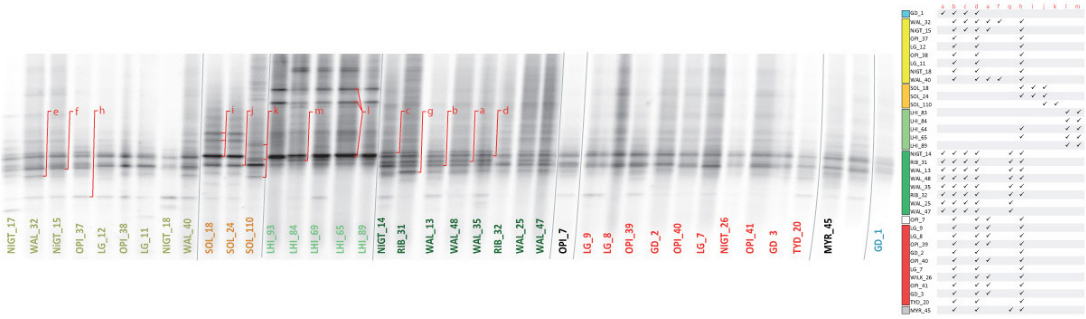


Figure S2.4: Left: DGGE of *Symbiodinium* ITS2; letters indicate diagnostic bands of each *Symbiodinium* type. Right: Table showing scoring results.

Table S2.1: Number of samples and location used for each of the data sets.

Location	ORF sequences produced for	CR, HSP70, ITS2 sequences produced for	*Symbiodinium* associations analysed of
Orpheus Island/Pelorus Island (OPI)	36	6	6
Wallace Islet Reef (WAL)	24	7	7
Great Keppel Island (KEP)	19	0	0
Wilkie Reef (WILK)	20	1	1
Tydeman Reef (TYD)	6	1	1
Rib Reef (RIB)	6	2	2
Night Reef (NIGT)	6	3	3
Long Reef (LG)	6	5	5
Great Detached Reef (GD)	6	3	3
Myrmidon Reef (MYR)	2	1	1
Lord Howe Island (LHI)	5	5	5
Solitary Islands (SOL)	3	3	3
One Tree Island (OTI)	6	0	0
TOTAL	**145**	**37**	**37**

MATERIALS OF CHAPTER 3

S3

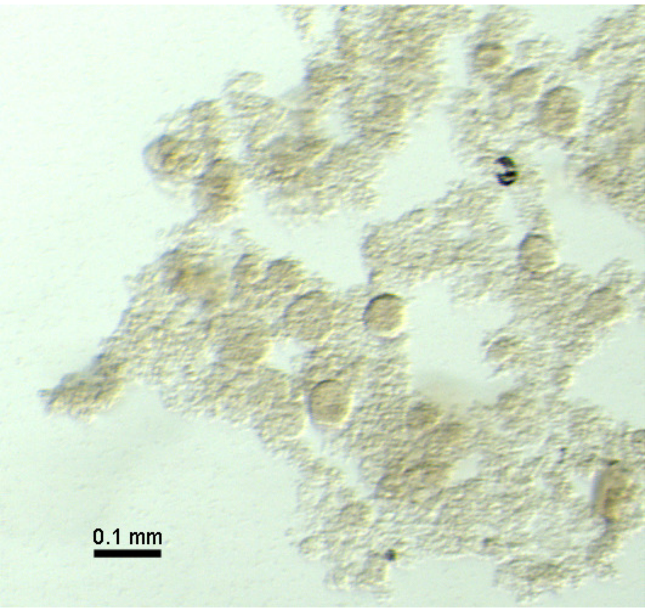


Figure S3.1: Eggs and sperm of *Pocillopora damicornis* preserved in ethanol

MATERIALS OF CHAPTER 5

S4

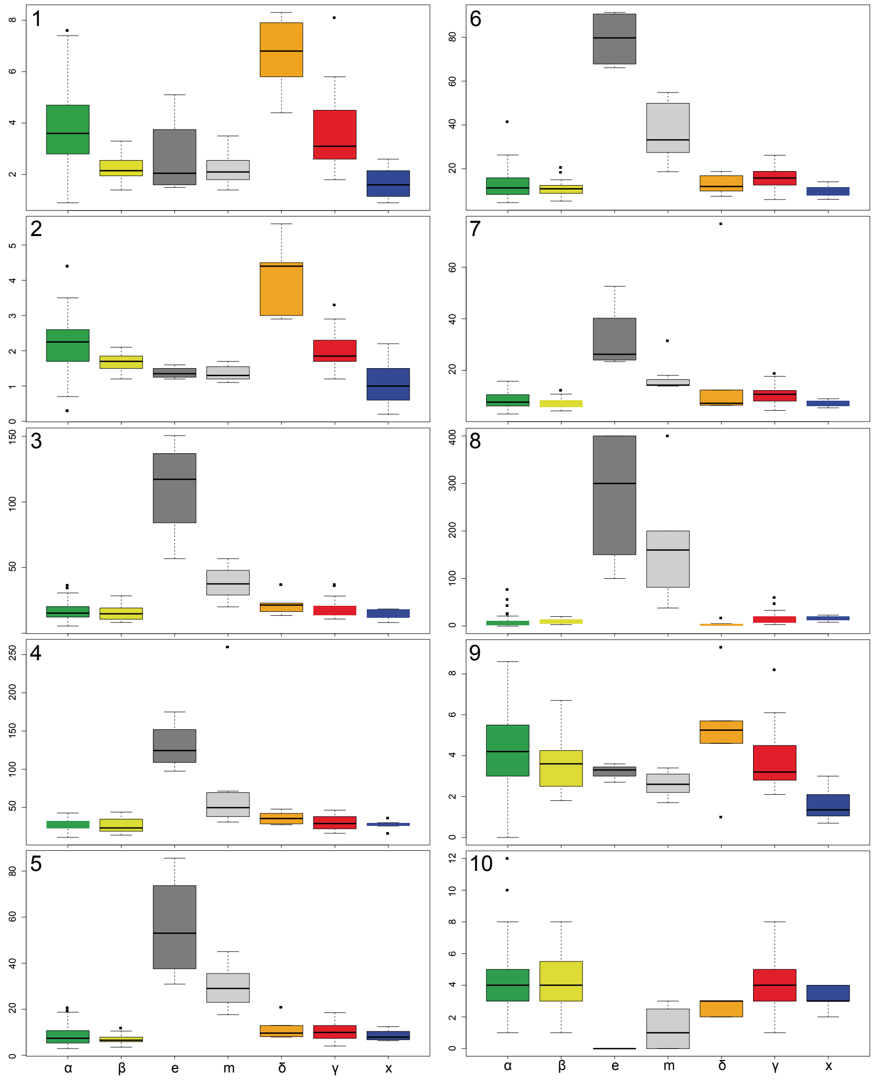
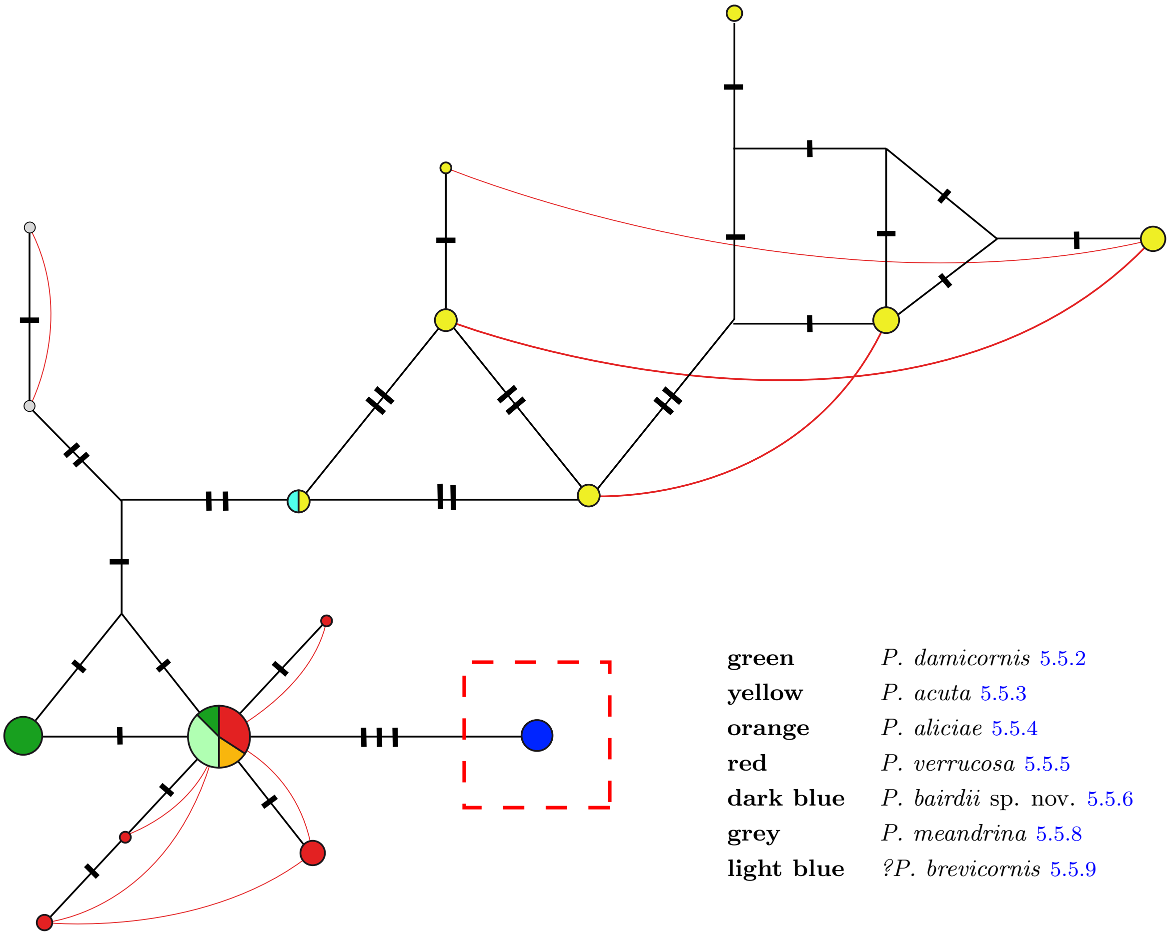


Figure S4.1: Box plots of morphological variables for each identified group, colors indicate genetic lineages labeled at the bottom. 1. The maximal diameter of most distal branchlet/verruca 1 mm under tip, at most distal branch. 2. Minimal diameter of most distal branchlet/verruca 1 mm under tip, at most distal branch. 3. Distance between most distal branch tip and the base of the most distal ramification of a main branch with secondary branching. 4. Distance between most distal branch tip and the base of the second most distal ramification of a main branch with secondary branching (or base of colony). 5. Maximal diameter half way between most distal branch tip and the base of the most distal ramification of a main branch with secondary branching. 6. Maximal diameter of branch at this most distal ramification of a main branch with secondary branching. 7. Minimal diameter of branch at this most distal ramification of a main branch with secondary branching. 8. Number of primary branches (branchlets/verrucae) between tip of most distal branch and most distal ramification of a main branch with secondary branching (with numbers exceeding 100 estimated to the closest decade). 9. Length of longest primary branch (branchlets/verrucae) between tip of most distal branch and most distal ramification of a main branch with secondary branching. 10. Number of branches with secondary (or higher) branching originating from investigated branch between tip and 4 cm below tip (or base of colony).



green	*P. damicornis* 5.5.2
yellow	*P. acuta* 5.5.3
orange	*P. aliciae* 5.5.4
red	*P. verrucosa* 5.5.5
dark blue	*P. bairdii* sp. nov. 5.5.6
grey	*P. meandrina* 5.5.8
light blue	*?P. brevicornis* 5.5.9

Figure S4.2: Haploweb of HSP70B region for *Pocillopora* spp.. Red box indicates the new identified species.